IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Applicant:	H. William BOSCH et al.
Title:	LIQUID DOSAGE COMPOSITIONS OF STABLE NANOPARTICULATE ACTIVE AGENTS
Appl. No.:	10/619,539
Filing Date:	7/16/2003
Examiner:	Susan T. Tran
Art Unit:	1615
Confirmation Number:	6324

APPEAL BRIEF

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Sir:

Under the provisions of 37 C.F.R. § 41.37, Appellants file herewith an appeal brief to initiate a new appeal. An appeal brief was previously filed on May 5, 2009, which was the basis for the Examiner to reopen the prosecution. The present appeal brief addresses the issues raised in the non-final Office Action dated September 8, 2009.

Pursuant to 37 C.F.R. § 41.20, the previously paid appeal fee will be applied to the present appeal. If this fee is deemed to be insufficient, authorization is hereby given to charge any deficiency (or credit any balance) to the undersigned deposit account 19-0741.

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STATUTES

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REAL PARTY IN INTEREST

The real party in interest in this appeal is Elan Pharma International, Ltd., which is the assignee of the present application as recorded at Reel/Frame numbers 015072/0837.

RELATED APPEALS AND INTERFERENCES

No related appeals or interferences are pending.

STATUS OF CLAIMS

Claims 4, 36, 38, 40, 42, 53 and 83 are canceled.

Claims 1-3, 5-35, 37, 39, 41, 43-52, 54-82, 84-123 are pending in the application, with claims 46-52, 54-82, 84-123 withdrawn from consideration. The claims under examination and the withdrawn claims are related as product and process claims. Therefore, the withdrawn process claims are subject to a rejoinder upon allowance of the corresponding product claims.

Claims 1-3, 5-35, 37, 39, 41, 43-45 are finally rejected, and are the subject of this appeal. The pending claims are presented in Appendix A of this Brief.

STATUS OF AMENDMENTS

No claim amendments were made in the response to final Office Action, filed on Decemher 18, 2008. In the final Offiee Aetion dated October 8, 2008, the Examiner indieated entry and eonsideration of an amendment filed July 3, 2008. No other amendments or submissions are pending in the application.

SUMMARY OF CLAIMED SUBJECT MATTER

Independent claim 1 is to be argued in the brief. The citation to the specification is shown in the parenthesis.

Independent claim 1 reads as follows:

1. A stable nanoparticulate liquid dosage composition **{p. 8, ll. 2-3, 24-25}** comprising:

(a) particles of at least one active agent having an effective average particle size of less than 2000 nm **{p. 8, ll. 3-4, 25-26; p. 30, ll. 5-7}**;

(b) at least one surface stabilizer **{p. 8, ll. 4, 26-27}**;

(c) at least one osmotically active crystal growth inhibitor **{p. 8, ll. 5, 27-28}** that is capable of preventing crystal growth of the active agent **{p. 28, ll. 15-17}** at ambient temperature **{p. 28, ll. 18-19}**, wherein the osmotically active crystal growth inhibitor is selected from the group consisting of glycerol **{p. 8, l. 7; p. 28, l. 18}**, propylene glycol **{p. 28, ll. 18-19}**, mannitol **{p. 8, l. 7; p. 28, l. 19}**, sucrose **{p. 28, l. 20}**, glucose **{p. 28, l. 20}**, fructose **{p. 28, l. 20}**, mannose **{p. 28, l. 20}**, lactose **{p. 28, l. 20}**, xylitol **{p. 28, l. 20}**, sorbitol **{p. 28, l. 20}**, trehalose **{p. 28, l. 20}**, a polysaccharide **{p. 28, l. 21}**, a mono-polysaccharide **{p. 28, l. 21}**, a di-polysaccharides **{p. 28, l. 21}**, a sugars **{p. 28, l. 21}**, a sugar alcohol **{p. 28, l. 21}**, sodium chloride **{p. 8, l. 7; p. 28, l. 22}**, potassium chloride **{p. 28, l. 22}**, magnesium chloride **{p. 28, l. 22}**, and an ionic salt **{p. 28, ll. 22-23}**; and

(d) a liquid media **{p. 8, l. 30}**,

wherein the liquid dosage composition does not incorporate a cloud point modifier **{p. 6, ll. 7-24; p. 7, ll. 24-29; p. 9, ll. 8-9}**.

GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

The first rejection to be reviewed on appeal is the rejection of claim 2 under 35 U.S.C. § 112, first paragraph, for alleged lack of enablement.

The second rejection to be reviewed on appeal is the rejection of claim 15 under 35 U.S.C. § 112, second paragraph, for allegedly failing to further limit the subject matter of claim 1.

The third rejection to be reviewed on appeal is the rejection of claims 1-3, 5, 6, 8-19, 21-24, 27-29, 32-35, 37, 39, 41 and 43-45 under 35 U.S.C. § 102(b) for alleged anticipation by U.S. Patent No. 5,302,401 to Liversidge et al. ("Liversidge '401").

The fourth rejection to be reviewed on appeal is the rejection of claims 1-3, 5-24, 26-31, 35, 37, 39, 41 and 43-45 under 35 U.S.C. § 102(e) for alleged anticipation by U.S. Patent Application No. 2003/0077329 by Kipp et al. ("Kipp").

The fifth rejection to be reviewed on appeal is the rejection of claims 1-3, 5-24, 27-29, 32-35, 37, 39, 41 and 43-45 under 35 U.S.C. § 103(a) over Liversidge '401, in view of PCT Application Publication No. WO 01/78505 by Brockbank et al. ("Brockbank") or Kipp.

The sixth rejection to be reviewed on appeal is the rejection of claims 25-35, 37, 39, 41 and 43-45 under 35 U.S.C. § 103(a) over Liversidge '401, in view of U.S. Patent Application Publication No. 2005/0004049 by Liversidge ("Liversidge '049").

ARGUMENT

I. Rejection of Claims under 35 U.S.C. §112

Rejection of claims 2 and 15 are collectively discussed in this section.

Both claim 2 and claim 15 were originally filed on July 16, 2003 and no subsequent claim amendments were made. In addition to the Office Action issued on September 8, 2009, four Office Actions have been issued, namely, the non-final Office Action issued on February 7, 2007, the final Office Action issued on September 20, 2007, the non-final Office Action issued on April 17, 2008, and the final Office Action issued on October 8, 2008. The 35 U.S.C. §112 rejections of claim 2 and claim 15 were not raised in any of the prior Office Actions until Appellants filed the first Appeal Brief on May 5, 2009. Accordingly, Appellants were not given a fair opportunity to submit claim amendments to place the claims in condition for allowance or at least in better condition for appeal.

Pursuant to M.P.E.P. 707.07(g), piecemeal examination should be avoided. The M.P.E.P. states: "Major technical rejections on grounds such as lack of proper disclosure, lack of enablement, serious indefiniteness and *res judicata* should be applied where appropriate even though there may be a seemingly sufficient rejection on the basis of prior art."

Appellants respectfully request that appropriate claim amendment, if necessary, be made by way of an Examiner's Amendment upon receipt of a favorable decision on the appeal from the Board.

II. Rejection over Liversidge '401

The claimed invention is directed to a ***stable*** nanoparticulate ***liquid dosage*** composition comprising a nanoparticulate active agent and at least one surface stabilizer, as well as at least one osmotically active crystal growth inhibitor.

As disclosed in the specification, a liquid dosage form is "particularly useful in treating patient populations such as the elderly, infants, and pediatrics" (page 8, lines 20-21). However, a liquid dosage form must be physically stable to be suitable for therapeutic uses. *See* page 4, last full paragraph. Nanoparticulate active agents present in liquid dosage form are prone to particle aggregation and crystal growth, which are undesirable and have a negative impact on the pharmacokinetic profiles of nanoparticulate active agent compositions. *See* page 4, line 29, through page 5, line 31. The claimed invention employs commonly used, non-toxic, osmotically active pharmaceutical ingredients to effectively reduce active agent particle aggregation and crystal growth, thereby obtaining a ***stable*** nanoparticulate active agent, ***liquid dosage*** composition without adverse effects. *See* page 6, third paragraph, and page 8, first paragraph.

In contrast to the claimed invention, Liversidge '401 is irrelevant to obtaining a stable nanoparticulate active agent liquid dosage composition. Rather, Liversidge '401 targets the problem of particle size growth during a ***lyophilization*** process. *See* column 1, lines 17-34. In other words, unlike the claimed ***liquid dosage*** composition, the nanoparticulate active agent composition of Liversidge '401 is in ***lyophilized dry powder form***.

More specifically, Liversidge '401 discloses contacting a nanoparticulate active agent composition with a cryoprotectant, which functions to prevent active agent particle size growth during lyophilization, and then allowing the composition to be lyophilized. *See* column 1, lines 36-49, and 56-58. Although cryoprotectants of Liversidge '401 encompass some pharmaceutical ingredients overlapping the osmotically active crystal growth inhibitors of the claimed invention, one skilled in the art would not have obtained the claimed liquid dosage based on the prior art's disclosure of a lyophilized composition.

Accordingly, Appellants respectfully request the Board to reverse the rejection over Liversidge '401.

III. Rejection over Kipp

As discussed *supra*, the claimed invention relates to a ***stable*** nanoparticulate active agent ***liquid dosage*** composition. Kipp discloses prolonging the storage of a suspension of drug particles "by encasing the drug particles in a ***frozen aqueous matrix***" because drug dissolution and degradation will be slowed down at low temperature and crystallization of water may help to prevent crystal growth of drug particles. Page 4, paragraph [0039]. Accordingly, Kipp does not teach or suggest stabilizing a nanoparticulate active agent liquid dosage composition by adding an osmotically active crystal growth inhibitor. Rather, Kipp solves the problem by storing the composition at freezing temperature so that the drug particles are "encased in a ***frozen aqueous matrix***." As such, Kipp does not teach a ***liquid dosage*** to anticipate the claimed invention. Reversal of the rejection in whole is respectfully requested.

IV. Rejection over Liversidge '401 and Brockbank or Kipp

The teachings of Liversidge '401 are discussed in the foregoing paragraphs. Brockbank and Kipp are cited for the alleged teaching of additional cryoprotectant compounds. Office Action, page 7, second full paragraph; and the paragraph bridging pages 7 and 8.

First, Liversidge '401 is directed to a lyophilized dry powder composition rather than a liquid dosage composition as in the claimed invention. Therefore, the alleged teachings of additional cryoproetectant compounds by the secondary references would not have cured the deficiencies of Liversidge '401.

Second, as discussed above, Liversidge '401 attempts to obtain a stable nanoparticulate active agent composition by lyophilizing the composition to prolong the storage in dry powder form. Although Kipp shares the common goal of obtaining a stable composition, Kipp achieves the goal by encasing the composition in a frozen aqueous matrix at low temperature. Accordingly, one skilled in the art would not have had any reason to combine the teachings of Liversidge '401 and Kipp because these references take entirely different approaches that would

have rendered the proposed modification impossible. Pursuant to M.P.E.P. 2143.01, "[i]f the proposed modification or combination of the prior art would change the principle of operation of the prior art invention being modified, then the teachings of the references are not sufficient to render the claims *prima facie* obvious."

V. Rejection over Liversidge '401 and Liversidge '049

The teachings of Liversidge '401 are discussed above. Liversidge '049 is cited for the alleged teaching of a bioadhesive nanoparticulate active agent composition. Nevertheless, Liversidge '049 does not compensate for the deficiencies of Liversidge '401. Therefore, the rejected dependent claims will stand or fall together with the base claim(s).

CONCLUSION

For the reasons discussed above, Appellants respectfully submit that all pending claims are in condition for allowance, and respectfully requests that the rejections be reversed in whole, and that the claims be allowed to issue.

Respectfully submitted,

Date: February 5, 2010

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APPENDIX A: CLAIMS INVOLVED IN APPEAL

1. (Previously Presented) A stable nanoparticulate liquid dosage composition comprising:

(a) particles of at least one active agent having an effective average particle size of less than 2000 nm;

(b) at least one surface stabilizer;

(c) at least one osmotically active crystal growth inhibitor that is capable of preventing crystal growth of the active agent at ambient temperature, wherein the osmotically active crystal growth inhibitor is selected from the group consisting of glycerol, propylene glycol, mannitol, sucrose, glucose, fructose, mannose, lactose, xylitol, sorbitol, trehalose, a polysaccharide, a mono-polysaccharide, a di-polysaccharides, a sugars, a sugar alcohol, sodium chloride, potassium chloride, magnesium chloride, and an ionic salt; and

(d) a liquid media,

wherein the liquid dosage composition does not incorporate a cloud point modifier.

2. (Original) The composition of claim 1, wherein the active agent particles form crystals upon storage or heating in the absence of the crystal growth inhibitor.

3. (Original) The composition of claim 1, wherein the osmotically active crystal growth inhibitor is at least partially water-soluble and does not solubilize the nanoparticulate active agent.

4. (Cancelled)

5. (Previously Presented) The composition of claim 1, wherein the crystal growth inhibitor is glycerol.

6. (Previously Presented) The composition of claim 1, where the crystal growth inhibitor is mannitol.

7. (Previously Presented) The composition of claim 1, where the crystal growth inhibitor is sodium chloride.

8. (Original) The composition of claim 1, wherein the amount of the crystal growth inhibitor present in the liquid dosage form ranges from about 0.1% to about 95% concentration, by weight.

9. (Original) The composition of claim 1, wherein the amount of the crystal growth inhibitor present in the liquid dosage form ranges from about 0.5% to about 90% concentration, by weight.

10. (Original) The composition of claim 1, wherein the effective average particle size of the nanoparticulate active agent particles is selected from the group consisting of less than about 1900 nm, less than about 1800 nm, less than about 1700 nm, less than about 1600 nm, less than about 1500 nm, less than about 1400 nm, less than about 1300 nm, less than about 1200 nm, less than about 1100 nm, less than about 1000 nm, less than about 900 nm, less than about 800 nm, less than about 700 nm, less than about 600 nm, less than about 500 nm, less than about 400 nm, less than about 300 nm, less than about 250 nm, less than about 200 nm, less than about 100 nm, less than about 75 nm, and less than about 50 nm.

11. (Original) The composition of claim 1 or 10, wherein at least about 70%, at least about 90%, or at least about 95% of the active agent particles have a particle size less than the effective average particle size.

12. (Original) The composition of claim 1, wherein the amount of the active agent per ml is equal to or greater than the amount of the active agent per ml of a standard conventional non-nanoparticulate liquid dosage composition of the same active agent.

13. (Original) The composition of claim 1, wherein the liquid media of the liquid dosage composition is selected from the group consisting of water, safflower oil, ethanol, t-butanol, glycerin, polyethylene glycol (PEG), hexane, and glycol.

14. (Original) The composition of claim 1, wherein the composition is formulated for administration selected from the group consisting of oral, pulmonary, rectal, ophthalmic, colonic, parenteral, intracisternal, intravaginal, intraperitoneal, local, buccal, nasal, and topical administration.

15. (Original) The composition of claim 1 formulated into a dosage form selected from the group consisting of liquid dispersions, oral suspensions, gels, aerosols, ointments, creams, controlled release formulations, fast melt formulations, lyophilized formulations, tablets, capsules, delayed release formulations, extended release formulations, pulsatile release formulations, and mixed immediate release and controlled release formulations.

16. (Original) The composition of claim 1, wherein the at least one active agent is present in an amount selected from the group consisting of from about 99.5% to about 0.001%, from about 95% to about 0.1%, and from about 90% to about 0.5%, by weight, based on the total combined weight of the active agent and at least one surface stabilizer, not including other excipients.

17. (Original) The composition of claim 1, wherein the at least one surface stabilizer is present in an amount selected from the group consisting of from about 0.5% to about 99.999% by weight, from about 5.0% to about 99.9% by weight, and from about 10% to about 99.5% by weight, based on the total combined dry weight of the active agent and at least one surface stabilizer, not including other excipients.

18. (Original) The composition of claim 1, wherein the ratio of active agent to a polymeric surface modifier is selected from the group consisting of from about 20:1 to about 1:10, from about 10:1 to about 1:5, and from about 5:1 to about 1:1, by weight.

19. (Original) The composition of claim 1, comprising at least two surface stabilizers.

20. (Original) The composition of claim 19, wherein the ratio of active agent to the second surface stabilizer is selected from the group consisting of from about 500:1 to about 5:1, from about 350:1 to about 10:1, and from about 100:1 to about 20:1, by weight.

21. (Original) The composition of claim 1, wherein the composition further comprises one or more pharmaceutically acceptable excipients, carriers, or a combination thereof.

22. (Original) The composition of claim 1, wherein the surface stabilizer is selected from the group consisting of an anionic surface stabilizer, a cationic surface stabilizer, a polymeric surface stabilizer, a nonionic surface stabilizer, and a zwitterionic surface stabilizer.

23. (Original) The composition of claim 22, wherein the at least one surface stabilizer is selected from the group consisting of cetyl pyridinium chloride, gelatin, casein, phosphatides, dextran, glycerol, gum acacia, cholesterol, tragacanth, stearic acid, benzalkonium chloride, calcium stearate, glycerol monostearate, cetostearyl alcohol, cetomacrogol emulsifying wax, sorbitan esters, polyoxyethylene alkyl ethers, polyoxyethylene castor oil derivatives, polyoxyethylene sorbitan fatty acid esters, polyethylene glycols, dodecyl trimethyl ammonium bromide, polyoxyethylene stearates, colloidal silicon dioxide, phosphates, sodium dodecylsulfate, carboxymethylcellulose calcium, hydroxypropyl celluloses, hypromellose, carboxymethylcellulose sodium, methylcellulose, hydroxyethylcellulose, hypromellose phthalate, noncrystalline cellulose, magnesium aluminum silicate, triethanolamine, polyvinyl alcohol, polyvinylpyrrolidone, 4-(1,1,3,3-tetramethylbutyl)-phenol polymer with ethylene oxide and formaldehyde, poloxamers; poloxamines, a charged phospholipid, dioctylsulfosuccinate, dialkylesters of sodium sulfosuccinic acid, sodium lauryl sulfate, alkyl aryl polyether sulfonates, mixtures of sucrose stearate and sucrose distearate, p-isononylphenoxypoly-(glycidol), decanoyl-N-methylglucamide; n-decyl β-D-glucopyranoside; n-decyl β-D-maltopyranoside; n-dodecyl β-

D-glucopyranoside; n-dodecyl β-D-maltoside; heptanoyl-N-methylglucamide; n-heptyl-β-D-glucopyranoside; n-heptyl β-D-thioglucoside; n-hexyl β-D-glucopyranoside; nonanoyl-N-methylglucamide; n-noyl β-D-glucopyranoside; octanoyl-N-methylglucamide; n-octyl-β-D-glucopyranoside; octyl β-D-thioglucopyranoside; lysozyme, PEG-phospholipid, PEG-cholesterol, PEG-cholesterol derivative, PEG-vitamin A, and random copolymers of vinyl acetate and vinyl pyrrolidonc.

24. (Previously Presented) The composition of claim 22, wherein the at least one cationic surface stabilizer is selected from the group consisting of a polymer, a biopolymer, a polysaccharide, a cellulosic, an alginate, a nonpolymeric compound, a phospholipid, cationic lipids, polymethylmethacrylate trimethylammonium bromide, sulfonium compounds, polyvinylpyrrolidone-2-dimethylaminoethyl methacrylate dimethyl sulfate, hexadecyltrimethyl ammonium bromide, phosphonium compounds, quarternary ammonium compounds, benzyl-di(2-chloroethyl)ethylammonium bromide, coconut trimethyl ammonium chloride, coconut trimethyl ammonium bromide, coconut methyl dihydroxyethyl ammonium chloride, coconut methyl dihydroxyethyl ammonium bromide, decyl triethyl ammonium chloride, decyl dimethyl hydroxyethyl ammonium chloride, decyl dimethyl hydroxyethyl ammonium chloride bromide, C_{12-15}dimethyl hydroxyethyl ammonium chloride, C_{12-15}dimethyl hydroxyethyl ammonium chloride bromide, coconut dimethyl hydroxyethyl ammonium chloride, coconut dimethyl hydroxyethyl ammonium bromide, myristyl trimethyl ammonium methyl sulphate, lauryl dimethyl benzyl ammonium chloride, lauryl dimethyl benzyl ammonium bromide, lauryl dimethyl $(ethenoxy)_4$ ammonium chloride, lauryl dimethyl $(ethenoxy)_4$ ammonium bromide, N-alkyl (C_{12-18})dimethylbenzyl ammonium chloride, N-alkyl (C_{14-18})dimethyl-benzyl ammonium chloride, N-tetradecylidmethylbenzyl ammonium chloride monohydrate, dimcthyl didecyl ammonium chloride, N-alkyl and (C_{12-14}) dimethyl 1-napthylmethyl ammonium chloride, trimethylammonium halide, alkyl-trimethylammonium salts, dialkyl-dimethylammonium salts, lauryl trimethyl ammonium

chloride, ethoxylated alkyamidoalkyldialkylammonium salt, an ethoxylated trialkyl ammonium salt, dialkylbenzene dialkylammonium chloride, N-didecyldimethyl ammonium chloride, N-tetradecyldimethylbenzyl ammonium, chloride monohydrate, N-alkyl(C_{12-14}) dimethyl 1-naphthylmethyl ammonium chloride, dodecyldimethylbenzyl ammonium chloride, dialkyl benzenealkyl ammonium chloride, lauryl trimethyl ammonium chloride, alkylbenzyl methyl ammonium chloride, alkyl benzyl dimethyl ammonium bromide, C_{12} trimethyl ammonium bromides, C_{15} trimethyl ammonium bromides, C_{17} trimethyl ammonium bromides, dodecylbenzyl triethyl ammonium chloride, poly-diallyldimethylammonium chloride (DADMAC), dimethyl ammonium chlorides, alkyldimethylammonium halogenides, tricetyl methyl ammonium chloride, decyltrimethylammonium bromide, dodecyltriethylammonium bromide, tetradecyltrimethylammonium bromide, methyl trioctylammonium chloride, polyquaternium 10, tetrabutylammonium bromide, benzyl trimethylammonium bromide, choline esters, benzalkonium chloride, stearalkonium chloride compounds, cetyl pyridinium bromide, cetyl pyridinium chloride, halide salts of quaternized polyoxyethylalkylamines, quaternized ammonium salt polymers, alkyl pyridinium salts; amines, amine salts, amine oxides, imide azolinium salts, protonated quaternary acrylamides, methylated quaternary polymers, and cationic guar.

25. (Original) The composition of any of claims 22 or 24, wherein the composition is bioadhesive.

26. (Original) The composition of claim 1, wherein the active agent is selected from the group consisting of a crystalline phase, an amorphous phase, a semi-crystalline phase, a semi-amorphous phase, and mixtures thereof.

27. (Original) The composition of claim 1, wherein the one or more active agents have a solubility in water selected from the group consisting of less than about 30 mg/ml, less

than about 20 mg/ml, less than about 10 mg/ml, and less than about 1 mg/ml, under ambient conditions.

28. (Original) The composition of claim 1 wherein the active agent comprises anti-inflammatory and analgesic properties.

29. (Original) The composition of claim 1, wherein the at least one active agent is selected from the group consisting of COX-2 inhibitors, anticancer agents, NSAIDS, proteins, peptides, nutraceuticals, anti-obesity agents, corticosteroids, elastase inhibitors, analgesics, anti-fungals, oncology therapies, anti-emetics, analgesics, cardiovascular agents, anti-inflammatory agents, anthelmintics, anti-arrhythmic agents, antibiotics, anticoagulants, antidepressants, antidiabetic agents, antiepileptics, antihistamines, antihypertensive agents, antimuscarinic agents, antimycobacterial agents, antineoplastic agents, immunosuppressants, antithyroid agents, antiviral agents, anxiolytics, sedatives, astringents, beta-adrenoceptor blocking agents, blood products and substitutes, cardiac inotropic agents, contrast media, cough suppressants, diagnostic agents, diagnostic imaging agents, diuretics, dopaminergics, haemostatics, immunological agents, lipid regulating agents, muscle relaxants, parasympathomimetics, parathyroid calcitonin and biphosphonates, prostaglandins, radio-pharmaceuticals, sex hormones, anti-allergic agents, stimulants and anoretics, sympathomimetics, thyroid agents, vasodilators, xanthines, acne medication, alpha-hydroxy formulations, cystic-fibrosis therapies, asthma therapies, emphysema therapies, respiratory distress syndrome therapies, chronic bronchitis therapies, chronic obstructive pulmonary disease therapies, organ-transplant rejection therapies, therapies for tuberculosis and other infections of the lung, and respiratory illness therapies associated with acquired immune deficiency syndrome.

30. (Original) The composition of claim 29, wherein the nutraceutical is selected from the group consisting of dietary supplements, vitamins, minerals, herbs, healing foods that have medical or pharmaceutical effects on the body, folic acid, fatty acids, fruit and vegetable extracts, vitamin supplements, mineral supplements, phosphatidylserine, lipoic acid, melatonin,

glucosamine/chondroitin, Aloe Vera, Guggul, glutamine, amino acids, green tea, lycopene, whole foods, food additives, herbs, phytonutrients, antioxidants, flavonoid constituents of fruits, evening primrose oil, flax seeds, fish and marine animal oils, and probiotics.

31. (Original) The composition of claim 1, wherein the active agent is selected from the group consisting of acyclovir, alprazolam, altretamine, amiloride, amiodarone, benztropine mesylate, bupropion, cabergoline, candesartan, cerivastatin, chlorpromazine, ciprofloxacin, cisapride, clarithromycin, clonidine, clopidogrel, cyclobenzaprine, cyproheptadine, delavirdine, desmopressin, diltiazem, dipyridamole, dolasetron, enalapril maleate, enalaprilat, famotidine, felodipine, furazolidone, glipizide, irbesartan, ketoconazole, lansoprazole, loratadine, loxapine, mebendazole, mercaptopurine, milrinone lactate, minocycline, mitoxantrone, nelfinavir mesylate, nimodipine, norfloxacin, olanzapine, omeprazole, penciclovir, pimozide, tacolimus, quazepam, raloxifene, rifabutin, rifampin, risperidone, rizatriptan, saquinavir, sertraline, sildenafil, acetyl-sulfisoxazole, temazepam, thiabendazole, thioguanine, trandolapril, triamterene, trimetrexate, troglitazone, trovafloxacin, verapamil, vinblastine sulfate, mycophenolate, atovaquone, atovaquone, proguanil, ceftazidime, cefuroxime, etoposide, terbinafine, thalidomide, fluconazole, amsacrine, dacarbazine, teniposide, and acetylsalicylate.

32. (Previously Presented) The composition of claim 1, having a viscosity, at a shear rate of 0.1 (1/s), is selected from the group consisting of from about 2000 mPa·s to about 1 mPa·s, from about 1900 mPa·s to about 1 mPa·s, from about 1800 mPa·s to about 1 mPa·s, from about 1700 mPa·s to about 1 mPa·s, from about 1600 mPa·s to about 1 mPa·s, from about 1500 mPa·s to about 1 mPa·s, from about 1400 mPa·s to about 1 mPa·s, from about 1300 mPa·s to about 1 mPa·s, from about 1200 mPa·s to about 1 mPa·s, from about 1100 mPa·s to about 1 mPa·s, from about 1000 mPa·s to about 1 mPa·s, from about 900 mPa·s to about 1 mPa·s, from about 800 mPa·s to about 1 mPa·s, from about 700 mPa·s to about 1 mPa·s, from about 600 mPa·s to about 1 mPa·s, from about 500 mPa·s to about 1 mPa·s, from about 400 mPa·s to about 1 mPa·s, from about 300 mPa·s to about 1 mPa·s, from about 200 mPa·s to about 1 mPa·s, from

about 175 mPa·s to about 1 mPa·s, from about 150 mPa·s to about 1 mPa·s, from about 125 mPa·s to about 1 mPa·s, from about 100 mPa·s to about 1 mPa·s, from about 75 mPa·s to about 1 mPa·s, from about 50 mPa·s to about 1 mPa·s, from about 25 mPa·s to about 1 mPa·s, from about 15 mPa·s to about 1 mPa·s, from about 10 mPa·s to about 1 mPa·s, and from about 5 mPa·s to about 1 mPa·s.

33. (Previously Presented) The composition of claim 1, having a viscosity selected from the group consisting of less than about 1/200, less than about 1/100, less than about 1/50, less than about 1/25, and less than about 1/10 of the viscosity of a standard conventional non-nanoparticulate liquid dosage composition of the same active agent at about the same concentration per ml of active agent.

34. (Previously Presented) The composition of claim 1, having a viscosity selected from the group consisting of less than about 5%, less than about 10%, less than about 15%, less than about 20%, less than about 25%, less than about 30%, less than about 35%, less than about 40%, less than about 45%, less than about 50%, less than about 55%, less than about 60%, less than about 65%, less than about 70%, less than about 75%, less than about 80%, less than about 85%, and less than about 90% of the viscosity of a standard conventional non-nanoparticulate liquid dosage composition of the same active agent at about the same concentration per ml of active agent.

35. (Previously Presented) The composition of claim 1, having a T_{max}, when assayed in the plasma of a mammalian subject following administration, less than the T_{max} for a conventional, non-nanoparticulate form of the same active agent, administered at the same dosage.

36. (Cancelled)

37. (Previously Presented) The composition of claim 1, having a C_{max}, when assayed in the plasma of a mammalian subject following administration, greater than the C_{max} for a

conventional, non-nanoparticulate form of the same active agent, administered at the same dosage.

38. (Cancelled)

39. (Previously Presented) The composition of claim 1, having an AUC, when assayed in the plasma of a mammalian subject following administration, greater than the AUC for a conventional, non-nanoparticulate form of the same active agent, administered at the same dosage.

40. (Cancelled)

41. (Original) The composition of claim 1 which does not produce significantly different absorption levels when administered under fed as compared to fasting conditions.

42. (Cancelled)

43. (Original) The composition of claim 1, wherein administration of the composition to a subject in a fasted state is bioequivalent to administration of the composition to a subject in a fed state, when administered to a human.

44. (Original) The composition of claim 43, wherein "bioequivalency" is established by a 90% Confidence Interval of between 0.80 and 1.25 for both C_{max} and AUC, when administered to a human.

45. (Original) The composition of claim 43, wherein "bioequivalency" is established by a 90% Confidence Interval of between 0.80 and 1.25 for AUC and a 90% Confidence Interval of between 0.70 to 1.43 for C_{max}, when administered to a human.

46. (Withdrawn) A method of making a stable nanoparticulate liquid dosage composition comprising contacting particles of at least one active agent with at least one surface

stabilizer in the presence of a liquid media for a time and under conditions sufficient to provide a nanoparticulate active agent composition wherein:

(a) the active agent particles have an effective average particle size of less than 2000 nm; and

(b) at least one osmotically active crystal growth inhibitor is added to the composition either before, during, or after the active agent particle size reduction, wherein the osmotically active crystal growth inhibitor is selected from the group consisting of glycerol, propylene glycol, mannitol, sucrose, glucose, fructose, mannose, lactose, xylitol, sorbitol, trehalose, a polysaccharide, a mono-polysaccharide, a di-polysaccharides, a sugars, a sugar alcohol, sodium chloride, potassium chloride, magnesium chloride, and an ionic salt.

47. (Withdrawn) The method of claim 46, wherein said contacting comprising grinding.

48. (Withdrawn) The method of claim 47, wherein said grinding comprising wet grinding.

49. (Withdrawn) The method of claim 46, wherein said contacting comprises homogenizing.

50. (Withdrawn) The method of claim 46, wherein said contacting comprises:

(a) dissolving the particles of at least one active agent in a solvent;

(b) adding the resulting solution of the active agent to a solution comprising at least one surface stabilizer; and

(c) precipitating the solubilized active agent and at least one surface stabilizer by the addition thereto of a non-solvent.

51. (Withdrawn) The method of claim 46, wherein the active agent particles form crystals upon storage or heating in the absence of the crystal growth inhibitor.

52. (Withdrawn) The method of claim 46, wherein the osmotically active crystal growth inhibitor is at least partially water-soluble and does not solubilize the nanoparticulate active agent.

53. (Cancelled)

54. (Withdrawn) The method of claim 46, wherein the crystal growth inhibitor is glycerol.

55. (Withdrawn) The method of claim 46, where the crystal growth inhibitor is mannitol.

56. (Withdrawn) The method of claim 46, where the crystal growth inhibitor is sodium chloride.

57. (Withdrawn) The method of claim 46, wherein the amount of the crystal growth inhibitor present in the liquid dosage composition ranges from about 0.1% to about 95% concentration, by weight.

58. (Withdrawn) The method of claim 57, wherein the amount of the crystal growth inhibitor present in the liquid dosage composition ranges from about 0.5% to about 90% concentration, by weight.

59. (Withdrawn) The method of claim 46, wherein the effective average particle size of the nanoparticulate active agent particles is selected from the group consisting of less than about 1900 nm, less than about 1800 nm, less than about 1700 nm, less than about 1600 nm, less than about 1500 nm, less than about 1400 nm, less than about 1300 nm, less than about 1200 nm, less than about 1100 nm, less than about 1000 nm, less than about 900 nm, less than about 800 nm, less than about 700 nm, less than about 600 nm, less than about 500 nm, less than about 400

nm, less than about 300 nm, less than about 250 nm, less than about 200 nm, less than about 100 nm, less than about 75 nm, and less than about 50 nm.

60. (Withdrawn) The method of claim 46 or 59, wherein at least about 70%, about 90%, or about 95% of the active agent particles have a particle size less than the effective average particle size.

61. (Withdrawn) The method of claim 46, wherein the liquid media of the liquid dosage composition is selected from the group consisting of water, safflower oil, ethanol, t-butanol, glycerin, polyethylene glycol (PEG), hexane, and glycol.

62. (Withdrawn) The method of claim 46, wherein the at least one active agent is present in an amount selected from the group consisting of from about 99.5% to about 0.001%, from about 95% to about 0.1%, and from about 90% to about 0.5%, by weight, based on the total combined weight of the active agent and at least one surface stabilizer, not including other excipients.

63. (Withdrawn) The method of claim 46, wherein the at least one surface stabilizer is present in an amount selected from the group consisting of from about 0.5% to about 99.999% by weight, from about 5.0% to about 99.9% by weight, and from about 10% to about 99.5% by weight, based on the total combined dry weight of the active agent and at least one surface stabilizer, not including other excipients.

64. (Withdrawn) The method of claim 46, wherein the ratio of active agent to a polymeric surface modifier is selected from the group consisting of from about 20:1 to about 1:10, from about 10:1 to about 1:5, and from about 5:1 to about 1:1, by weight.

65. (Withdrawn) The method of claim 46, comprising at least two surface stabilizers.

66. (Withdrawn) The method of claim 65, wherein the ratio of active agent to the second surface stabilizer is selected from the group consisting of from about 500:1 to about 5:1, from about 350:1 to about 10:1, and from about 100:1 to about 20:1, by weight.

67. (Withdrawn) The method of claim 46, wherein the surface stabilizer is selected from the group consisting of an anionic surface stabilizer, a cationic surface stabilizer, a polymeric surface stabilizer, a nonionic surface stabilizer, and a zwitterionic surface stabilizer.

68. (Withdrawn) The method of claim 67, wherein the at least one surface stabilizer is selected from the group consisting of cetyl pyridinium chloride, gelatin, casein, phosphatides, dextran, glycerol, gum acacia, cholesterol, tragacanth, stearic acid, benzalkonium chloride, calcium stearate, glycerol monostearate, cetostearyl alcohol, cetomacrogol emulsifying wax, sorbitan esters, polyoxyethylene alkyl ethers, polyoxyethylene castor oil derivatives, polyoxyethylene sorbitan fatty acid esters, polyethylene glycols, dodecyl trimethyl ammonium bromide, polyoxyethylene stearates, colloidal silicon dioxide, phosphates, sodium dodecylsulfate, carboxymethylcellulose calcium, hydroxypropyl celluloses, hypromellose, carboxymethylcellulose sodium, methylcellulose, hydroxyethylcellulose, hypromellose phthalate, noncrystalline cellulose, magnesium aluminum silicate, triethanolamine, polyvinyl alcohol, polyvinylpyrrolidone, 4-(1,1,3,3-tetramethylbutyl)-phenol polymer with ethylene oxide and formaldehyde, poloxamers; poloxamines, a charged phospholipid, dioctylsulfosuccinate, dialkylesters of sodium sulfosuccinic acid, sodium lauryl sulfate, alkyl aryl polyether sulfonates, mixtures of sucrose stearate and sucrose distearate, p-isononylphenoxypoly-(glycidol), decanoyl-N-methylglucamide; n-decyl β-D-glucopyranoside; n-decyl β-D-maltopyranoside; n-dodecyl β-D-glucopyranoside; n-dodecyl β-D-maltoside; heptanoyl-N-methylglucamide; n-heptyl-β-D-glucopyranoside; n-heptyl β-D-thioglucoside; n-hexyl β-D-glucopyranoside; nonanoyl-N-methylglucamide; n-noyl β-D-glucopyranoside; octanoyl-N-methylglucamide; n-octyl-β-D-glucopyranoside; octyl β-D-thioglucopyranoside; lysozyme, PEG-phospholipid, PEG-cholesterol,

PEG-cholesterol derivative, PEG-vitamin A, and random copolymers of vinyl acetate and vinyl pyrrolidone.

69. (Withdrawn) The method of claim 67, wherein the at least one cationic surface stabilizer is selected from the group consisting of a polymer, a biopolymer, a polysaccharide, a cellulosic, an alginate, a nonpolymeric compound, a phospholipid, cationic lipids, polymethylmethacrylate trimethylammonium bromide, sulfonium compounds, polyvinylpyrrolidone-2-dimethylaminoethyl methacrylate dimethyl sulfate, hexadecyltrimethyl ammonium bromide, phosphonium compounds, quarternary ammonium compounds, benzyl-di(2-ehloroethyl)ethylammonium bromide, eoeonut trimethyl ammonium chloride, coconut trimethyl ammonium bromide, coconut methyl dihydroxyethyl ammonium ehloride, coconut methyl dihydroxyethyl ammonium bromide, decyl triethyl ammonium ehloride, decyl dimethyl hydroxyethyl ammonium ehloride, decyl dimethyl hydroxyethyl ammonium ehloride bromide, C_{12-15}dimethyl hydroxyethyl ammonium chloride, C_{12-15}dimethyl hydroxyethyl ammonium ehloride bromide, coconut dimethyl hydroxyethyl ammonium ehloride, coconut dimethyl hydroxyethyl ammonium bromide, myristyl trimethyl ammonium methyl sulphate, lauryl dimethyl benzyl ammonium chloride, lauryl dimethyl benzyl ammonium bromide, lauryl dimethyl $(ethenoxy)_4$ ammonium chloride, lauryl dimethyl $(ethenoxy)_4$ ammonium bromide, N-alkyl (C_{12-18})dimethylbenzyl ammonium ehloride, N-alkyl (C_{14-18})dimethyl-benzyl ammonium chloride, N-tetradecylidmethylbenzyl ammonium ehloride monohydrate, dimethyl didecyl ammonium chloride, N-alkyl and (C_{12-14}) dimethyl 1-napthylmethyl ammonium chloride, trimethylammonium halide, alkyl-trimethylammonium salts, dialkyl-dimethylammonium salts, lauryl trimethyl ammonium chloride, ethoxylated alkyamidoalkyldialkylammonium salt, an ethoxylated trialkyl ammonium salt, dialkylbenzene dialkylammonium chloride, N-didecyldimethyl ammonium chloride, N-tetradecyldimethylbenzyl ammonium, chloride monohydrate, N-alkyl(C_{12-14}) dimethyl 1-naphthylmethyl ammonium chloride, dodecyldimethylbenzyl ammonium chloride, dialkyl

benzenealkyl ammonium chloride, lauryl trimethyl ammonium chloride, alkylbenzyl methyl ammonium chloride, alkyl benzyl dimethyl ammonium bromide, C_{12} trimethyl ammonium bromides, C_{15} trimethyl ammonium bromides, C_{17} trimethyl ammonium bromides, dodecylbenzyl triethyl ammonium chloride, poly-diallyldimethylammonium chloride (DADMAC), dimethyl ammonium chlorides, alkyldimethylammonium halogenides, tricctyl methyl ammonium chloride, decyltrimethylammonium bromide, dodecyltriethylammonium bromide, tetradecyltrimethylammonium bromide, methyl trioctylammonium chloride, polyquaternium 10, tetrabutylammonium bromide, benzyl trimethylammonium bromide, choline esters, benzalkonium chloride, stearalkonium chloride compounds, cetyl pyridinium bromide, cetyl pyridinium chloride, halide salts of quaternized polyoxyethylalkylamines, quaternized ammonium salt polymers, alkyl pyridinium salts; amines, amine salts, amine oxides, imide azolinium salts, protonated quaternary acrylamides, methylated quaternary polymers, and cationic guar.

70. (Withdrawn) The method of claim 46, wherein the active agent is selected from the group consisting of a crystalline phase, an amorphous phase, and a semi-crystalline phase.

71. (Withdrawn) The method of claim 46, wherein the one or more active agents have a solubility in water selected from the group consisting of less than about 30 mg/ml, less than about 20 mg/ml, less than about 10 mg/ml, and less than about 1 mg/ml, under ambient conditions.

72. (Withdrawn) The method of claim 46, wherein the active agent comprises anti-inflammatory and analgesic properties.

73. (Withdrawn) The method of claim 46, wherein the at least one active agent is selected from the group consisting of COX-2 inhibitors, anticancer agents, NSAIDS, proteins, peptides, nutraceuticals, anti-obesity agents, corticosteroids, elastase inhibitors, analgesics, anti-fungals, oncology therapies, anti-emetics, analgesics, cardiovascular agents, anti-inflammatory

agents, anthelmintics, anti-arrhythmic agents, antibiotics, anticoagulants, antidepressants, antidiabetic agents, antiepileptics, antihistamines, antihypertensive agents, antimuscarinic agents, antimycobacterial agents, antineoplastic agents, immunosuppressants, antithyroid agents, antiviral agents, anxiolytics, sedatives, astringents, beta-adrenoceptor blocking agents, blood products and substitutes, cardiac inotropic agents, contrast media, cough suppressants, diagnostic agents, diagnostic imaging agents, diuretics, dopaminergics, haemostatics, immunological agents, lipid regulating agents, muscle relaxants, parasympathomimetics, parathyroid calcitonin and biphosphonates, prostaglandins, radio-pharmaceuticals, sex hormones, anti-allergic agents, stimulants and anoretics, sympathomimetics, thyroid agents, vasodilators, xanthines, acne medication, alpha-hydroxy formulations, cystic-fibrosis therapies, asthma therapies, emphysema therapies, respiratory distress syndrome therapies, chronic bronchitis therapies, chronic obstructive pulmonary disease therapies, organ-transplant rejection therapies, therapies for tuberculosis and other infections of the lung, and respiratory illness therapies associated with acquired immune deficiency syndrome.

74. (Withdrawn) The method of claim 73, wherein the nutraceutical is selected from the group consisting of dietary supplements, vitamins, minerals, herbs, healing foods that have medical or pharmaceutical effects on the body, folic acid, fatty acids, fruit and vegetable extracts, vitamin supplements, mineral supplements, phosphatidylserine, lipoic acid, melatonin, glucosamine/chondroitin, Aloe Vera, Guggul, glutamine, amino acids, green tea, lycopene, whole foods, food additives, herbs, phytonutrients, antioxidants, flavonoid constituents of fruits, evening primrose oil, flax seeds, fish and marine animal oils, and probiotics.

75. (Withdrawn) The method of claim 46, wherein the active agent is selected from the group consisting of acyclovir, alprazolam, altretamine, amiloride, amiodarone, benztropine mesylate, bupropion, cabergoline, candesartan, cerivastatin, chlorpromazine, ciprofloxacin, cisapride, clarithromycin, clonidine, clopidogrel, cyclobenzaprine, cyproheptadine, delavirdine, desmopressin, diltiazem, dipyridamole, dolasetron, enalapril maleate, enalaprilat, famotidine,

felodipine, furazolidone, glipizide, irbesartan, ketoconazole, lansoprazole, loratadine, loxapine, mebendazole, mercaptopurine, milrinone lactate, minocycline, mitoxantrone, nelfinavir mesylate, nimodipine, norfloxacin, olanzapine, omeprazole, penciclovir, pimozide, tacolimus, quazepam, raloxifene, rifabutin, rifampin, risperidone, rizatriptan, saquinavir, sertraline, sildenafil, acetyl-sulfisoxazole, temazepam, thiabendazole, thioguanine, trandolapril, triamterene, trimetrexate, troglitazone, trovafloxacin, verapamil, vinblastine sulfate, mycophenolate, atovaquone, atovaquone, proguanil, ceftazidime, cefuroxime, etoposide, terbinafine, thalidomide, fluconazole, amsacrine, dacarbazine, teniposide, and acetylsalicylate.

76. (Withdrawn) A method of treating a subject with a stable nanoparticulate liquid dosage composition comprising administering to the subject an effective amount of a composition comprising:

(a) particles of at least one active agent having an effective average particle size of less than 2000 nm;

(b) at least one surface stabilizer;

(c) at least one osmotically active crystal growth inhibitor, wherein the osmotically active crystal growth inhibitor is selected from the group consisting of glycerol, propylene glycol, mannitol, sucrose, glucose, fructose, mannose, lactose, xylitol, sorbitol, trehalose, a polysaccharide, a mono-polysaccharide, a di-polysaccharides, a sugars, a sugar alcohol, sodium chloride, potassium chloride, magnesium chloride, and an ionic salt; and

(d) a liquid media.

77. (Withdrawn) The method of claim 76, wherein said subject is a human.

78. (Withdrawn) The method of claim 76, wherein the condition to be treated is selected from the group consisting of neoplastic diseases, breast cancer, endometrial cancer, uterine cancer, cervical cancer, prostate cancer, renal cancer, hormone replacement therapy in post-menopausal women, endometriosis, hirsutism, dysmenorrhea, uterine bleeding, HIV wasting, cancer wasting, migraine headache, cachexia, anorexia, castration, oral contraception,

motion sickness, emesis related to cytotoxic drugs, gastritis, ulcers, dyspepsia, gastroenteritis, including collitis and food poisoning, inflammatory bowel disease, Crohn's disease, migraine headaches, and any other condition which is accompanied by the symptoms of nausea and vomiting.

79. (Withdrawn) The method of claim 76, wherein the condition to be treated is selected from the group consisting of pain, inflammation, arthritis, cancer, kidney disease, osteoporosis, Alzheimer's disease, and familial adenomatous polyposis.

80. (Withdrawn) The method of claim 79, wherein the condition to be treated is selected from the group consisting of osteoarthritis, rheumatoid arthritis, juvenile arthritis, gout, ankylosing spondylitis, systemic lupus erythematosus, bursitis, tendinitis, myofascial pain, carpal tunnel syndrome, fibromyalgia syndrome, infectious arthritis, psoriatic arthritis, reiter's syndrome, and scleroderma.

81. (Withdrawn) The method of claim 76, wherein the active agent particles form crystals upon storage or heating in the absence of the crystal growth inhibitor.

82. (Withdrawn) The method of claim 76, wherein the osmotically active crystal growth inhibitor is at least partially water-soluble and does not solubilize the nanoparticulate active agent.

83. (Cancelled)

84. (Withdrawn) The method of claim 76, wherein the crystal growth inhibitor is glycerol.

85. (Withdrawn) The method of claim 76, where the crystal growth inhibitor is mannitol.

86. (Withdrawn) The method of claim 76, where the crystal growth inhibitor is sodium chloride.

87. (Withdrawn) The method of claim 76, wherein the amount of the crystal growth inhibitor present in the liquid dosage composition ranges from about 0.1% to about 95% concentration, by weight.

88. (Withdrawn) The method of claim 76, wherein the amount of the crystal growth inhibitor present in the liquid dosage composition ranges from about 0.5% to about 90% concentration, by weight.

89. (Withdrawn) The method of claim 76, wherein the effective average particle size of the nanoparticulate active agent particles is selected from the group consisting of less than about 1900 nm, less than about 1800 nm, less than about 1700 nm, less than about 1600 nm, less than about 1500 nm, less than about 1400 nm, less than about 1300 nm, less than about 1200 nm, less than about 1100 nm, less than about 1000 nm, less than about 900 nm, less than about 800 nm, less than about 700 nm, less than about 600 nm, less than about 500 nm, less than about 400 nm, less than about 300 nm, less than about 250 nm, less than about 200 nm, less than about 100 nm, less than about 75 nm, and less than about 50 nm.

90. (Withdrawn) The method of claim 76 or 89, wherein at least about 70%, about 90%, or about 95% of the active agent particles have a particle size less than the effective average particle size.

91. (Withdrawn) The method of claim 76, wherein the amount of the active agent per ml is equal to or greater than the amount of the active agent per ml of a standard conventional non-nanoparticulate liquid dosage composition of the same active agent.

92. (Withdrawn) The method of claim 76, wherein the liquid media of the liquid dosage composition is selected from the group consisting of water, safflower oil, ethanol, t-butanol, glycerin, polyethylene glycol (PEG), hexane, and glycol.

93. (Withdrawn) The method of claim 76, wherein the composition is formulated for administration selected from the group consisting of oral, pulmonary, rectal, ophthalmic, colonic, parenteral, intracisternal, intravaginal, intraperitoneal, local, buccal, nasal, and topical administration.

94. (Withdrawn) The method of claim 76 formulated into a dosage form selected from the group consisting of liquid dispersions, oral suspensions, gels, aerosols, ointments, creams, controlled release formulations, fast melt formulations, lyophilized formulations, tablets, capsules, delayed release formulations, extended release formulations, pulsatile release formulations, and mixed immediate release and controlled release formulations.

95. (Withdrawn) The method of claim 76, wherein the at least one active agent is present in an amount selected from the group consisting of from about 99.5% to about 0.001%, from about 95% to about 0.1%, and from about 90% to about 0.5%, by weight, based on the total combined weight of the active agent and at least one surface stabilizer, not including other excipients.

96. (Withdrawn) The method of claim 76, wherein the at least one surface stabilizer is present in an amount selected from the group consisting of from about 0.5% to about 99.999% by weight, from about 5.0% to about 99.9% by weight, and from about 10% to about 99.5% by weight, based on the total combined dry weight of the active agent and at least one surface stabilizer, not including other excipients.

97. (Withdrawn) The method of claim 76, wherein the ratio of active agent to a polymeric surface modifier is selected from the group consisting of from about 20:1 to about 1:10, from about 10:1 to about 1:5, and from about 5:1 to about 1:1, by weight.

98. (Withdrawn) The method of claim 76, comprising at least two surface stabilizers.

99. (Withdrawn) The method of claim 98, wherein the ratio of active agent to the second surface stabilizer is selected from the group consisting of from about 500:1 to about 5:1, from about 350:1 to about 10:1, and from about 100:1 to about 20:1, by weight.

100. (Withdrawn) The method of claim 76, wherein the composition further comprises one or more pharmaceutically acceptable excipients, carriers, or a combination thereof.

101. (Withdrawn) The method of claim 76, wherein the surface stabilizer is selected from the group consisting of an anionic surface stabilizer, a cationic surface stabilizer, a polymeric surface stabilizer, a nonionic surface stabilizer, and a zwitterionic surface stabilizer.

102. (Withdrawn) The method of claim 101, wherein the at least one surface stabilizer is selected from the group consisting of cetyl pyridinium chloride, gelatin, casein, phosphatides, dextran, glycerol, gum acacia, cholesterol, tragacanth, stearic acid, benzalkonium chloride, calcium stearate, glycerol monostearate, cetostearyl alcohol, cetomacrogol emulsifying wax, sorbitan esters, polyoxyethylene alkyl ethers, polyoxyethylene castor oil derivatives, polyoxyethylene sorbitan fatty acid esters, polyethylene glycols, dodecyl trimethyl ammonium bromide, polyoxyethylene stearates, colloidal silicon dioxide, phosphates, sodium dodecylsulfate, carboxymethylcellulose calcium, hydroxypropyl celluloses, hypromellose, carboxymethylcellulose sodium, methylcellulose, hydroxyethylcellulose, hypromellose phthalate, noncrystalline cellulose, magnesium aluminum silicate, triethanolamine, polyvinyl alcohol, polyvinylpyrrolidone, 4-(1,1,3,3-tetramethylbutyl)-phenol polymer with ethylene oxide and formaldehyde, poloxamers; poloxamines, a charged phospholipid, dioctylsulfosuccinate, dialkylesters of sodium sulfosuccinic acid, sodium lauryl sulfate, alkyl aryl polyether sulfonates, mixtures of sucrose stearate and sucrose distearate, p-isononylphenoxypoly-(glycidol), decanoyl-N-methylglucamide; n-decyl β-D-glucopyranoside; n-decyl β-D-maltopyranoside; n-dodecyl β-D-glucopyranoside; n-dodecyl β-D-maltoside; heptanoyl-N-methylglucamide; n-heptyl-β-D-

glucopyranoside; n-heptyl β-D-thioglucoside; n-hexyl β-D-glucopyranoside; nonanoyl-N-methylglucamide; n-noyl β-D-glucopyranoside; octanoyl-N-methylglucamide; n-octyl-β-D-glucopyranoside; octyl β-D-thioglucopyranoside; lysozyme, PEG-phospholipid, PEG-cholesterol, PEG-cholesterol derivative, PEG-vitamin A, and random copolymers of vinyl acetate and vinyl pyrrolidone.

103. (Withdrawn) The method of claim 101, wherein the at least one cationic surface stabilizer is selected from the group consisting of a polymer, a biopolymer, a polysaccharide, a cellulosic, an alginate, a nonpolymeric compound, a phospholipid, cationic lipids, polymethylmethacrylate trimethylammonium bromide, sulfonium compounds, polyvinylpyrrolidone-2-dimethylaminoethyl methacrylate dimethyl sulfate, hexadecyltrimethyl ammonium bromide, phosphonium compounds, quarternary ammonium compounds, benzyl-di(2-chloroethyl)ethylammonium bromide, coconut trimethyl ammonium chloride, coconut trimethyl ammonium bromide, coconut methyl dihydroxyethyl ammonium chloride, coconut methyl dihydroxyethyl ammonium bromide, decyl triethyl ammonium chloride, decyl dimethyl hydroxyethyl ammonium chloride, decyl dimethyl hydroxyethyl ammonium chloride bromide, C_{12-15}dimethyl hydroxyethyl ammonium chloride, C_{12-15}dimethyl hydroxyethyl ammonium chloride bromide, coconut dimethyl hydroxyethyl ammonium chloride, coconut dimethyl hydroxyethyl ammonium bromide, myristyl trimethyl ammonium methyl sulphate, lauryl dimethyl benzyl ammonium chloride, lauryl dimethyl benzyl ammonium bromide, lauryl dimethyl $(ethenoxy)_4$ ammonium chloride, lauryl dimethyl $(ethenoxy)_4$ ammonium bromide, N-alkyl (C_{12-18})dimethylbenzyl ammonium chloride, N-alkyl (C_{14-18})dimethyl-benzyl ammonium chloride, N-tetradecylidmethylbenzyl ammonium chloride monohydrate, dimethyl didecyl ammonium chloride, N-alkyl and (C_{12-14}) dimethyl 1-napthylmethyl ammonium chloride, trimethylammonium halide, alkyl-trimethylammonium salts, dialkyl-dimethylammonium salts, lauryl trimethyl ammonium chloride, ethoxylated alkyamidoalkyldialkylammonium salt, an ethoxylated trialkyl ammonium salt, dialkylbenzene dialkylammonium chloride, N-didecyldimethyl ammonium chloride, N-tetradecyldimethylbenzyl ammonium, chloride

monohydrate, N-alkyl(C_{12-14}) dimethyl 1-naphthylmethyl ammonium chloride, dodecyldimethylbenzyl ammonium chloride, dialkyl benzenealkyl ammonium chloride, lauryl trimethyl ammonium chloride, alkylbenzyl methyl ammonium chloride, alkyl benzyl dimethyl ammonium bromide, C_{12} trimethyl ammonium bromides, C_{15} trimethyl ammonium bromides, C_{17} trimethyl ammonium bromides, dodecylbenzyl triethyl ammonium chloride, poly-diallyldimethylammonium chloride (DADMAC), dimethyl ammonium chlorides, alkyldimethylammonium halogenides, tricetyl methyl ammonium chloride, decyltrimethylammonium bromide, dodecyltriethylammonium bromide, tetradecyltrimethylammonium bromide, methyl trioctylammonium chloride, polyquaternium 10, tetrabutylammonium bromide, benzyl trimethylammonium bromide, choline esters, benzalkonium chloride, stearalkonium chloride compounds, cetyl pyridinium bromide, cetyl pyridinium chloride, halide salts of quaternized polyoxyethylalkylamines, quaternized ammonium salt polymers, alkyl pyridinium salts; amines, amine salts, amine oxides, imide azolinium salts, protonated quaternary acrylamides, methylated quaternary polymers, and cationic guar.

104. (Withdrawn) The method of claim 76, wherein the active agent is selected from the group consisting of a crystalline phase, an amorphous phase, and a semi-crystalline phase.

105. (Withdrawn) The method of claim 76, wherein the one or more active agents have a solubility in water selected from the group consisting of less than about 30 mg/ml, less than about 20 mg/ml, less than about 10 mg/ml, and less than about 1 mg/ml, under ambient conditions.

106. (Withdrawn) The method of claim 76, wherein the active agent comprises anti-inflammatory and analgesic properties.

107. (Withdrawn) The method of claim 76, wherein the at least one active agent is selected from the group consisting of COX-2 inhibitors, anticancer agents, NSAIDS, proteins,

peptides, nutraceuticals, anti-obesity agents, corticosteroids, elastase inhibitors, analgesics, anti-fungals, oncology therapies, anti-emetics, analgesics, cardiovascular agents, anti-inflammatory agents, anthelmintics, anti-arrhythmic agents, antibiotics, anticoagulants, antidepressants, antidiabetic agents, antiepileptics, antihistamines, antihypertensive agents, antimuscarinic agents, antimycobacterial agents, antineoplastic agents, immunosuppressants, antithyroid agents, antiviral agents, anxiolytics, sedatives, astringents, beta-adrenoceptor blocking agents, blood products and substitutes, cardiac inotropic agents, contrast media, cough suppressants, diagnostic agents, diagnostic imaging agents, diuretics, dopaminergics, haemostatics, immunological agents, lipid regulating agents, muscle relaxants, parasympathomimetics, parathyroid calcitonin and biphosphonates, prostaglandins, radio-pharmaceuticals, sex hormones, anti-allergic agents, stimulants and anoretics, sympathomimetics, thyroid agents, vasodilators, xanthines, acne medication, alpha-hydroxy formulations, cystic-fibrosis therapies, asthma therapies, emphysema therapies, respiratory distress syndrome therapies, chronic bronchitis therapies, chronic obstructive pulmonary disease therapies, organ-transplant rejection therapies, therapies for tuberculosis and other infections of the lung, and respiratory illness therapies associated with acquired immune deficiency syndrome.

108. (Withdrawn) The method of claim 107, wherein the nutraceutical is selected from the group consisting of dietary supplements, vitamins, minerals, herbs, healing foods that have medical or pharmaceutical effects on the body, folic acid, fatty acids, fruit and vegetable extracts, vitamin supplements, mineral supplements, phosphatidylserine, lipoic acid, melatonin, glucosamine/chondroitin, Aloe Vera, Guggul, glutamine, amino acids, green tea, lycopene, whole foods, food additives, herbs, phytonutrients, antioxidants, flavonoid constituents of fruits, evening primrose oil, flax seeds, fish and marine animal oils, and probiotics.

109. (Withdrawn) The method of claim 76, wherein the active agent is selected from the group consisting of acyclovir, alprazolam, altretamine, amiloride, amiodarone, benztropine mesylate, bupropion, cabergoline, candesartan, cerivastatin, chlorpromazine, ciprofloxacin,

cisapride, clarithromycin, clonidine, clopidogrel, cyclobenzaprine, cyproheptadine, delavirdine, desmopressin, diltiazem, dipyridamole, dolasetron, enalapril maleate, enalaprilat, famotidine, felodipine, furazolidone, glipizide, irbesartan, ketoconazole, lansoprazole, loratadine, loxapine, mebendazole, mercaptopurine, milrinone lactate, minocycline, mitoxantrone, nelfinavir mesylate, nimodipine, norfloxacin, olanzapine, omeprazole, penciclovir, pimozide, tacolimus, quazepam, raloxifene, rifabutin, rifampin, risperidone, rizatriptan, saquinavir, sertraline, sildenafil, acetyl-sulfisoxazole, temazepam, thiabendazole, thioguanine, trandolapril, triamterene, trimetrexate, troglitazone, trovafloxacin, verapamil, vinblastine sulfate, mycophenolate, atovaquone, atovaquone, proguanil, ceftazidime, cefuroxime, etoposide, terbinafine, thalidomide, fluconazole, amsacrine, dacarbazine, teniposide, and acetylsalicylate.

110. (Withdrawn) The method of claim 76, wherein the viscosity of the composition, at a shear rate of 0.1 (1/s), is selected from the group consisting of from about 2000 mPa·s to about 1 mPa·s, from about 1900 mPa·s to about 1 mPa·s, from about 1800 mPa·s to about 1 mPa·s, from about 1700 mPa·s to about 1 mPa·s, from about 1600 mPa·s to about 1 mPa·s, from about 1500 mPa·s to about 1 mPa·s, from about 1400 mPa·s to about 1 mPa·s, from about 1300 mPa·s to about 1 mPa·s, from about 1200 mPa·s to about 1 mPa·s, from about 1100 mPa·s to about 1 mPa·s, from about 1000 mPa·s to about 1 mPa·s, from about 900 mPa·s to about 1 mPa·s, from about 800 mPa·s to about 1 mPa·s, from about 700 mPa·s to about 1 mPa·s, from about 600 mPa·s to about 1 mPa·s, from about 500 mPa·s to about 1 mPa·s, from about 400 mPa·s to about 1 mPa·s, from about 300 mPa·s to about 1 mPa·s, from about 200 mPa·s to about 1 mPa·s, from about 175 mPa·s to about 1 mPa·s, from about 150 mPa·s to about 1 mPa·s, from about 125 mPa·s to about 1 mPa·s, from about 100 mPa·s to about 1 mPa·s, from about 75 mPa·s to about 1 mPa·s, from about 50 mPa·s to about 1 mPa·s, from about 25 mPa·s to about 1 mPa·s, from about 15 mPa·s to about 1 mPa·s, from about 10 mPa·s to about 1 mPa·s, and from about 5 mPa·s to about 1 mPa·s.

111. (Withdrawn) The method of claim 76, wherein the viscosity of the composition is selected from the group consisting of less than about 1/200, less than about 1/100, less than about 1/50, less than about 1/25, and less than about 1/10 of the viscosity of a standard conventional non-nanoparticulate liquid dosage composition of the same active agent at about the same concentration per ml of active agent.

112. (Withdrawn) The method of claim 76, wherein the viscosity of the composition is selected from the group consisting of less than about 5%, less than about 10%, less than about 15%, less than about 20%, less than about 25%, less than about 30%, less than about 35%, less than about 40%, less than about 45%, less than about 50%, less than about 55%, less than about 60%, less than about 65%, less than about 70%, less than about 75%, less than about 80%, less than about 85%, and less than about 90% of the viscosity of a standard conventional non-nanoparticulate liquid dosage composition of the same active agent at about the same concentration per ml of active agent.

113. (Withdrawn) The method of claim 76, wherein the T_{max} of the active agent, when assayed in the plasma of a mammalian subject following administration, is less than the T_{max} for a conventional, non-nanoparticulate form of the same active agent, administered at the same dosage.

114. (Withdrawn) The method of claim 113, wherein the T_{max} is selected from the group consisting of not greater than about 90%, not greater than about 80%, not greater than about 70%, not greater than about 60%, not greater than about 50%, not greater than about 30%, not greater than about 25%, not greater than about 20%, not greater than about 15%, and not greater than about 10% of the T_{max}, exhibited by a non-nanoparticulate formulation of the same active agent, administered at the same dosage.

115. (Withdrawn) The method of claim 76, wherein the C_{max} of the active agent, when assayed in the plasma of a mammalian subject following administration, is greater than the C_{max}

for a conventional, non-nanoparticulate form of the same active agent, administered at the same dosage.

116. (Withdrawn) The method of claim 115, wherein the C_{max} is selected from the group consisting of at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, and at least about 100% greater than the C_{max} exhibited by a non-nanoparticulate formulation of the same active agent, administered at the same dosage.

117. (Withdrawn) The method of claim 76, wherein the AUC of the active agent, when assayed in the plasma of a mammalian subject following administration, is greater than the AUC for a conventional, non-nanoparticulate form of the same active agent, administered at the same dosage.

118. (Withdrawn) The method of claim 117, wherein the AUC is selected from the group consisting of at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, and at least about 100% greater than the AUC exhibited by a non-nanoparticulate formulation of the same active agent, administered at the same dosage.

119. (Withdrawn) The method of claim 76 which does not produce significantly different absorption levels when administered under fed as compared to fasting conditions.

120. (Withdrawn) The method of claim 119, wherein the difference in absorption of the active agent composition of the invention, when administered in the fed versus the fasted state, is selected from the group consisting of less than about 100%, less than about 90%, less than about 80%, less than about 70%, less than about 60%, less than about 50%, less than about 40%, less than about 30%, less than about 25%, less than about 20%, less than about 15%, less than about 10%, less than about 5%, and less than about 3%.

121. (Withdrawn) The method of claim 76, wherein administration of the composition to a subject in a fasted state is bioequivalent to administration of the composition to a subject in a fed state, when administered to a human.

122. (Withdrawn) The method of claim 121, wherein "bioequivalency" is established by a 90% Confidence Interval of between 0.80 and 1.25 for both C_{max} and AUC, when administered to a human.

123. (Withdrawn) The method of claim 121, wherein "bioequivalency" is established by a 90% Confidence Interval of between 0.80 and 1.25 for AUC and a 90% Confidence Interval of between 0.70 to 1.43 for C_{max}, when administered to a human.

APPENDIX B: EVIDENCE

1. U.S. Patent No. 5,302,401 to Liversidge et al.;

2. U.S. Patent Application No. 2003/0077329 by Kipp et al.;

3. PCT Application Publication No. WO 01/78505 by Brockbank et al.; and

4. U.S. Patent Application Publication No. 2005/0004049 by Liversidge.

APPENDIX C: RELATED PROCEEDINGS

No related proceedings are pending.

US005302401A

United States Patent [19]

Liversidge et al.

[11] Patent Number: 5,302,401

[45] Date of Patent: Apr. 12, 1994

[54] METHOD TO REDUCE PARTICLE SIZE GROWTH DURING LYOPHILIZATION

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[58] Field of Search 424/9, 489, 499, 501; 514/171, 777, 788, 951

[56] References Cited

U.S. PATENT DOCUMENTS

5,145,684 9/1992 Liversidge et al. 424/489

FOREIGN PATENT DOCUMENTS

0193208 9/1986 European Pat. Off. .

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[57] ABSTRACT

This invention discloses a composition comprised of nanoparticles having a surface modifier adsorbed on the surface thereof and a cryoprotectant associated therewith, which cryoprotectant is present in an amount sufficient to form a nanoparticle-cryoprotectant composition. A preferred surface modifier is polyvinylpyrrolidone, and a preferred cryoprotectant is a carbohydrate such as sucrose. This invention further discloses a method of making nanoparticles having a surface modifier adsorbed on the surface and a cryoprotectant associated therewith, comprised of contacting said nanoparticles with the cryoprotectant for a time and under conditions sufficient to form a nanoparticle-cryoprotectant composition.

4 Claims, No Drawings

METHOD TO REDUCE PARTICLE SIZE GROWTH DURING LYOPHILIZATION

FIELD OF THE INVENTION

This invention relates to therapeutic and diagnostic compositions with a cryoprotectant, and to a method for the preparation thereof.

BACKGROUND OF THE INVENTION

Nanoparticles, described in U.S. Pat. No. 5,145,684, are particles consisting of a poorly soluble therapeutic or diagnostic agent onto which are adsorbed a non-crosslinked surface modifier, and which have an average particle size of less than about 400 nanometers (nm).

As a result of their small size, lyophilization of therapeutic and diagnostic agents in nanoparticulate form stabilized by a surface modifier (surfactant) is difficult. Conventional lyophilization results in substantial growth of particle size, rendering the resulting particles unusable thus losing the desirable properties provided by rapid dissolution of small drug particles. The present invention describes the application of lyophilization to preparation of freeze-dried drug nanoparticles that retain their small particle size and can be readily redispersed.

This invention is directed to novel compositions that allow lyophilization of nanoparticles with reduced or no particle size growth. These compositions provide for an addition of cryoprotectants to nanoparticles such that the nanoparticles do not agglomerate during lyophilization. This invention is also directed to a method of making such compositions.

SUMMARY OF THE INVENTION

This invention is directed to a composition comprised of nanoparticles having a surface modifier adsorbed on the surface thereof and a cryoprotectant associated therewith, which cryoprotectant is present in an amount sufficient to allow the nanoparticles to be lyophilized.

This invention further discloses a method of making nanoparticles having a surface modifier adsorbed on the surface thereof and a cryoprotectant associated therewith, said method comprising contacting said nanoparticles with the cryoprotectant for a time and under conditions sufficient to allow the nanoparticles to be lyophilized.

DETAILED DESCRIPTION OF THE INVENTION

This invention is directed to a composition comprised of nanoparticles having a surface modifier adsorbed on the surface thereof and a cryoprotectant associated therewith, which cryoprotectant is present in an amount sufficient to allow the nanoparticles to be lyophilized.

The nanoparticles useful in the practice of this invention include a surface modifier. Surface modifiers useful herein physically adhere to the surface of the x-ray contrast agent but do not chemically react with the agent or itself. Individually adsorbed molecules of the surface modifier are essentially free of intermolecular crosslinkages. Suitable surface modifiers can be selected from known organic and inorganic pharmaceutical excipients such as various polymers, low-molecular weight oligomers, natural products and surfactants. Preferred surface modifiers include nonionic and anionic surfactants.

Representative examples of surface modifiers include gelatin, casein, lecithin (phosphatides), gum acacia, cholesterol, tragacanth, stearic acid, benzalkonium chloride, calcium stearate, glyceryl monostearate, cetostearyl alcohol, cetomacrogol emulsifying wax, sorbitan esters, polyoxyethylene alkyl ethers, e.g., macrogol ethers such as cetomacrogol 1000, polyoxyethylene castor oil derivatives, polyoxyethylene sorbitan fatty acid esters, e.g., the commercially available Tweens™, polyethylene glycols, polyoxyethylene stearates, colloidal silicon dioxide, phosphates, sodium dodecylsulfate, carboxymethylcellulose calcium, carboxymethylcellulose sodium, methylcellulose, hydroxyethylcellulose, hydroxypropylcellulose, hydroxypropylmethylcellulose phthalate, noncrystalline cellulose, magnesium aluminum silicate, triethanolamine, polyvinyl alcohol, and polyvinylpyrrolidone (PVP). Most of these surface modifiers are known pharmaceutical excipients and are described in detail in the *Handbook of Pharmaceutical Excipients*, published jointly by the American Pharmaceutical Association and The Pharmaceutical Society of Great Britain, the Pharmaceutical Press, 1986.

Preferred surface modifiers include polyvinylpyrrolidone, tyloxapol, poloxamers such as Pluronic™ F68 and F108, which are block copolymers of ethylene oxide and propylene oxide, and poloxamines such as Tetronic™ 908 (also known as Poloxamine 908), which is a tetrafunctional block copolymer derived from sequential addition of propylene oxide and ethylene oxide to ethylenediamine, available from BASF, dextran, lecithin, dialkylesters of sodium sulfosuccinic acid, such as Aerosol OT™, which is a dioctyl ester of sodium sulfosuccinic acid, available from American Cyanamid, Duponol™ P, which is a sodium lauryl sulfate, available from DuPont, Triton™ X-200, which is an alkyl aryl polyether sulfonate, available from Rohm and Haas, Tween 80, which is a polyoxyethylene sorbitan fatty acid ester, available from ICI Specialty Chemicals, and Carbowax™ 3350 and 934, which are polyethylene glycols available from Union Carbide. Surface modifiers which have been found to be particularly useful include Tetronic 908, the Tweens™, Pluronic F-68 and polyvinylpyrrolidone. A particularly preferred surface modifier is polyvinylpyrrolidone (PVP). Other useful surface modifiers include:

decanoyl-N-methylglucamide;
n-decyl β-D-glucopyranoside;
n-decyl β-D-maltopyranoside;
n-dodecyl β-D-glucopyranoside;
n-dodecyl β-D-maltoside;
heptanoyl-N-methylglucamide
n-heptyl β-D-glucopyranoside;
n-heptyl β-D-thioglucoside;
n-hexyl β-D-glucopyranoside;
nonanoyl-N-methylglucamide;
n-nonyl β-D-glucopyranoside;
octanoyl-N-methylglucamide;
n-octyl β-D-glucopyranoside;
octyl β-D-thioglucopyranoside;
and the like.

The surface modifiers are commercially available and/or can be prepared by techniques known in the art. Two or more surface modifiers can be used in combination.

The nanoparticles useful in the practice of this invention can be prepared according to the methods disclosed in U.S. Pat. No. 5,145,684, whose disclosure is incorporated herein by reference. Briefly, nanoparticles are prepared by dispersing a poorly soluble therapeutic or diagnostic agent in a liquid dispersion medium and wet-grinding the agent in the presence of grinding media to reduce the particle size of the contrast agent to an effective average particle size of less than about 400 nm. The particles can be reduced in size in the presence of a surface modifier.

A general procedure for preparing the particles useful in the practice of this invention follows. The therapeutic or diagnostic agent selected is obtained commercially and/or prepared by techniques known in the art as described above, in a conventional coarse form. It is preferred, but not essential, that the particle size of the coarse therapeutic or diagnostic substance selected be less than about 100 μm as determined by sieve analysis. If the coarse particle size of that agent is greater than about 100 μm, then it is preferred that the coarse particles of the therapeutic or diagnostic agent be reduced in size to less than 100 μm using a conventional milling method such as airjet or fragmentation milling.

The coarse therapeutic or diagnostic agent selected can then be added to a liquid medium in which it is essentially insoluble to form a premix. The concentration of the therapeutic or diagnostic agent in the liquid medium can vary from about 0.1-60%, and preferably is from 5-30% (w/w). It is preferred, but not essential, that the surface modifier be present in the premix. The concentration of the surface modifier can vary from about 0.1 to 90%, and preferably is 1-75%, more preferably 10-60% and most preferably 10-30% by weight based on the total combined weight of the drug substance and surface modifier. The apparent viscosity of the premix suspension is preferably less than about 1000 centipoise.

The premix can be used directly by wet grinding to reduce the average particle size in the dispersion to less than 400 nm. It is preferred that the premix be used directly when a ball mill is used for attrition. Alternatively, the therapeutic or diagnostic agent and, optionally, the surface modifier, can be dispersed in the liquid medium using suitable agitation, e.g., a roller mill or a Cowles type mixer, until a homogeneous dispersion is observed in which there are no large agglomerates visible to the naked eye. It is preferred that the premix be subjected to such a premilling dispersion step when a recirculating media mill is used for attrition.

Wet grinding can take place in any suitable dispersion mill, including, for example, a ball mill, an attritor mill, a vibratory mill, and media mills such as a sand mill and a bead mill. A media mill is preferred due to the relatively shorter milling time required to provide the intended result, i.e., the desired reduction in particle size. For media milling, the apparent viscosity of the premix preferably is from about 100 to about 1000 centipoise. For ball milling, the apparent viscosity of the premix preferably is from about 1 up to about 100 centipoise. Such ranges tend to afford an optimal balance between efficient particle fragmentation and media erosion.

The grinding media for the particle size reduction step can be selected from rigid media preferably spherical or particulate in form having an average size less than about 3 mm and, more preferably, less than about 1 mm. Such media desirably can provide the particles of the invention with shorter processing times and impart less wear to the milling equipment. The selection of material for the grinding media is not believed to be critical. However,, preferred media have a density greater than about 3 g/cm^3. Zirconium oxide, such as 95% ZrO stabilized with magnesia, zirconium silicate, and glass grinding media provide particles having levels of contamination which are believed to be acceptable for the preparation of therapeutic or diagnostic compositions. However, other media, such as stainless steel, titania, alumina, and 95% ZrO stabilized with yttrium, are believed to be useful.

The attrition time can vary widely and depends primarily upon the particular wet grinding mill selected. For ball mills, processing times of up to five days or longer may be required. On the other hand, processing times of less than 1 day (residence times of about one minute up to several hours) have provided the desired results using a high shear media mill.

The particles must be reduced in size at a temperature which does not significantly degrade the therapeutic or diagnostic agent. Processing temperatures of less than about 30°-40° C. are ordinarily preferred. If desired, the processing equipment can be cooled with conventional cooling equipment. The method is conveniently carried out under conditions of ambient temperature and at processing pressures which are safe and effective for the milling process. For example, ambient processing pressures are typical of ball mills, attritor mills and vibratory mills. Processing pressures up to about 20 psi (1.4 kg/cm^2) are typical of media milling.

The surface modifier, if not present in the premix, must be added to the dispersion after attrition in an amount as described for the premix. Thereafter, the dispersion can be mixed, e.g., by shaking vigorously. Optionally, the dispersion can be subjected to a sonication step, e.g., using an ultrasonic power supply. For example, the dispersion can be subjected to ultrasonic energy having a frequency of 20-80 kHz for a time of about 1 to 120 seconds.

The relative amount of therapeutic or diagnostic agent and surface modifier can vary widely and the optimal amount of the surface modifier can depend, for example, upon the particular therapeutic or diagnostic agent and surface modifier selected, the critical micelle concentration of the surface modifier if it forms micelles, the hydrophilic lipophilic balance (HLB) of the stabilizer, the melting point of the stabilizer, its water solubility, the surface tension of water solutions of the stabilizer, etc. The surface modifier preferably is present in an amount of about 0.1-10 mg per square meter surface area of the therapeutic or diagnostic agent. The surface modifier can be present in an amount of 0.1-90%, preferably 1-75%, more preferably 10-60%, and most preferably 10-30% by weight based on the total weight of the dry particle.

Therapeutic and diagnostic agents useful in the composition of the present invention include those disclosed in U.S. Pat. No. 5,145,684, whose disclosure is incorporated herein by reference. A preferred therapeutic agent is 17-α-pregno-2,4-dien-20-yno-[2,3-d]-isoxazol-17-ol (Danazol).

As used herein, particle size refers to a number average particle size as measured by conventional particle size measuring techniques well known to those skilled in the art, such as sedimentation field flow fractionation, photon correlation spectroscopy, or disk centrifugation. By "an effective average particle size of less than about 400 nm" it is meant that at least 90% of the particles

have a weight average particle size of less than about 400 nm when measured by the above-noted techniques. In preferred embodiments of the invention, the effective average particle size is less than about 300 nm, and more preferably less than about 250 nm. In some embodiments of the invention, an effective average particle size of less than about 200 nm has been achieved. With reference to the effective average particle size, it is preferred that at least 95% and, more preferably, at least 99% of the particles have a particle size less than the effective average, e.g., 400 nm. In particularly preferred embodiments, essentially all of the particles have a size less than 400 nm. In some embodiments, essentially all of the particles have a size less than 250 nm.

Lyophilization is the process of freeze-drying a composition to remove excess water. The process involves the sublimation of the frozen water, usually under reduced pressure conditions. The process is well known in the art of lyophilization.

Cryoprotectants (cryoprotective agents or compounds) are agents that protect chemical compounds, cells, or tissues from the deleterious effects of freezing that may accompany lyophilization. In the case of nanoparticles, cryoprotectants protect from the agglomeration caused by the process of lyophilization, namely freeze-drying.

Exemplary cryoprotectants include carbohydrates such as the saccharide sucrose, sugar alcobols such as mannitol, surface active agents such as the Tweens, as well as glycerol and dimethylsulfoxide. A preferred cryoprotectant is a carbohydrate. A preferred carbohydrate is a saccharide or disaccharide. A preferred disaccharide is sucrose.

Cryoprotectants are present in the nanoparticles of the present invention in an amount sufficient to allow the nanoparticles to be lyophilized. Cryoprotectants are present in an amount of 0.5% to 90%, preferably 1-50%, and most preferably in an amount of about 2% to about 25%, based on the total weight of the nanoparticulate suspension.

A method for the preparation of a nanoparticle composition according to this invention includes the steps of introducing a therapeutic or diagnostic agent, a liquid medium, grinding media, and optionally, a surface modifier into a grinding vessel; wet grinding to reduce the particle size of the therapeutic or diagnostic agent to less than about 400 nm; and separating the particles and optionally the liquid medium from the grinding vessel and grinding media, for example, by suction, filtration or evaporation. If the surface modifier is not present during wet grinding, it can be admixed with the particles thereafter. The liquid medium, most often water, can serve as the pharmaceutically acceptable carrier. The method preferably is generally carried out under aseptic conditions.

This invention further discloses a method of making nanoparticles having a surface modifier adsorbed on the surface and a cryoprotectant associated therewith, comprised of contacting said nanoparticles with the cryoprotectant for a time and under conditions sufficient to form a nanoparticle-cryoprotectant composition. That composition allows the nanoparticles to be lyophilized.

This method involves the preparation of therapeutic or diagnostic nanoparticles, as discussed elsewhere herein, and contacting those nanoparticles with a cryoprotectant. Contacting may be by admixing a suspension of nanoparticles with a solution of cryoprotectant, followed by lyophilization at a temperature and for a time sufficient to effect freeze-drying of the nanoparticle suspension.

The amount of cryoprotectant used in this method is from about 0.5% to about 90%, preferably 1% to 50%, and most preferably from about 2% to about 25% based on the total weight of the nanoparticle suspension.

The following examples further illustrate the invention and are not to be construed as limiting of the specification and claims in any way.

EXAMPLE 1

Nanoparticulate Danazol Dispersions

A nanoparticulate dispersion of Danazol was lyophilized alone and in the presence of various cryoprotectants, namely Tween 80, mannitol and sucrose. The mean particle diameter was initially 185 nm. Unmodified dispersion (1 ml) was lyophilized and reconstituted in the initial volume of water. The resulting dispersion had a mean particle size of 250 nm. The original and reconstituted dispersions were analyzed using a HIAC/ROYCO instrument. The results revealed a significant increase in the number of particles above 10 μm in the reconsituted dispersion (>17,500 vs. <2000 in the original dispersion).

Addition of 0.02% Tween 80 or 2% mannitol to the original dispersion, followed by significantly lyophillization and reconstitution, did not significantly decrease the number of larger particles in the reconsituted dispersion. However, addition of 2% sucrose to the initial dispersion led to significantly less particles above 10 μm (21 7500) in the reconstituted dispersion.

EXAMPLE 2

A solution of Danazol (5% w/w) and polyvinylpyrrolidone (PVP) (1.5% w/w), along with 1.5% and 6.5% PVP alone were used in the experiments described in this Example.

From those three solutions above, a number of 6 mL vials with 1 mL of each (about 10 vials of each solution) were filled and prepared for freeze-drying according to the following cycle:

Prefreezing: +5° C. for one hour.
Freezing: Lower shelf temperature to −45° C. and hold for 2 hours.
Primary Drying: Engage vacuum at 80 microns and raise shelf temperature to −30° C. Hold at these conditions for 16 hours.
Secondary Drying: Decrease vacuum to 40 microns and increase shelf temperature to +25° C. Hold for 7 hours.

The vials were then stoppered automatically inside the dryer.

Upon visual examination of the reconstituted solutions, large particles were observed in all of the vials. Therefore, it was deduced that Danazol with PVP and PVP alone at different concentrations would form large particles or aggregates with these formulations and cycle conditions.

EXAMPLE 3

The samples of Example 2 were further modified with excipients such as a) those that reduce surface tension (surfactants; i.e., Tween 80), b) bulking agents (mannitol) and c) cryoprotectants (sucrose). The freeze-drying cycle was also modified.

Using this strategy, vials were loaded as in the previous Example with:

1) 5% w/w Danazol/1.5 w/w PVP (neat)

2) 1+0.02% w/v Tween 80

3) 1+2% w/v Mannitol

4) 1+2% w/v Sucrose

Modifications to the freeze-drying cycle consisted of 1) increasing the duration of freezing from +5° C. to −45° C. from 1 hour to 1.5 hours and 2) all other cycle parameters remained the same.

Upon reconstitution, it was observed that the sucrose containing solution appeared most like the starting material. The others did not. Therefore, the addition of sucrose to the Danazol/PVP solution accomplished what the other excipients could not.

EXAMPLE 4

The following solutions were prepared according to the methods in the above Examples.

1) Danazol (5%), 1.5% PVP (unlyophilized)

2) Danazol (5%), 1.5% PVP (freeze dried)

3) Danazol (5%), 1.5% PVP (freeze dried-2)

4) Danazol (5%), 1.5% PVP, 0.02% Tween 80

5) Danazol (5%), 1.5% PVP, 2% Mannitol

6) Danazol (5%), 1.5% PVP, 2% Sucrose

After lyophilization (except for Sample 1), the samples were analyzed by HIAC/Royco. The results are in Table 1. The data presented represents the average number of particles counted having a mean particle above 10 μm, 30 μm, 80 μm and 100 μm, respectively.

TABLE 1

	μm (x, n = 3)			
	10	30	80	100
1	1122	15	0	0
2	19801	978	2	0
3	17063	2153	1	0
4	18148	3071	2	0
5	19196	77	0	0
6	6368	94	0	0

The results indicate that the addition of sucrose to the Danazol/PVP solution substantially reduced particle size growth during lyophilization.

The foregoing specification, including the specific embodiments and examples is intended to be illustrative of the present invention and is not to be taken as limiting. Numerous other variations and modifications can be effected without departing from the true spirit and scope of the present invention.

We claim:

1. A composition comprised of nanoparticles containing 0.1-90% by weight of the therapeutic agent 17α-pregno-2,4-dien-20-yno-[2,3-d]-isoxazol-17-ol and having 99.9-10% by weight polyvinylpyrrolidone as a surface modifier adsorbed on the surface thereof and sucrose as the cryoprotectant associated therewith, which cryoprotectant is present in an amount of 0.5-90% by weight based on the total weight of the composition and sufficient to allow said nanoparticles to be lyophilized.

2. A method of making the composition of claim 1 comprised of contacting said nanoparticles with said cryoprotectant.

3. The method of claim 2 further comprising the step of lyophilizing said nanoparticle-cryoprotectant composition.

4. The method of claim 3 wherein said lyophilization is by freeze-drying.

* * * * *

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Kipp et al. (43) **Pub. Date: Apr. 24, 2003**

(54) **COMPOSITION OF AND METHOD FOR PREPARING STABLE PARTICLES IN A FROZEN AQUEOUS MATRIX**

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(57) **ABSTRACT**

The present invention discloses a composition of a stable suspension of a poorly water soluble pharmaceutical agent or cosmetic in the form of particles of the pharmaceutical agent or cosmetic suspended in a frozen aqueous matrix and method for its preparation. The composition is stable for a prolonged period of time, preferably six months or longer and is suitable for parenteral, oral, or non-oral routes such as pulmonary (inhalation), ophthalmic, or topical administration.

COMPOSITION OF AND METHOD FOR PREPARING STABLE PARTICLES IN A FROZEN AQUEOUS MATRIX

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority from provisional Application Serial No. 60/347,548 filed Oct. 19, 2001, which is incorporated herein by reference and made a part hereof.

FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT:

[0002] Not Applicable.

BACKGROUND OF THE INVENTION

[0003] 1. Technical Field

[0004] The present invention discloses a composition of a stable suspension of a poorly water soluble compound comprising particles of the compound suspended in a frozen aqueous matrix and method for its preparation. The composition is stable for a prolonged period of time, preferably six months or longer.

[0005] 2. Background Art

[0006] There is an ever increasing number of pharmaceutical compounds being formulated that are poorly soluble or insoluble in aqueous solutions. Such compounds provide challenges to delivering them in an injectable form. Drugs that are insoluble in water can have significant benefits when formulated as a stable suspension of sub-micron particles. Accurate control of particle size is essential for safe and efficacious use of these formulations. Particles must be less than seven microns in diameter to safely pass through capillaries without causing emboli (Allen et al., 1987; Davis and Taube, 1978; Schroeder et al., 1978; Yokel et al, 1981). One solution to this problem is the production of extremely small particles of the insoluble drug candidate and the creation of a microparticulate or nanoparticulate suspension. In this way, drugs that were previously unable to be formulated in an aqueous based system can be made suitable for intravenous administration. Suitability for intravenous administration includes small particle size (<7 μm), low toxicity (as from toxic formulation components or residual solvents), and bioavailability of the drug particles after administration.

[0007] Suspensions may also be suitable for oral, intramuscular, pulmonary, topical or subcutaneous administration. When administered by these routes, it may be desirable to have particle size in the range of 5 to 100 microns.

[0008] Suspensions may lack sufficient physical and chemical stability when stored for a prolonged period of time. Physical instability occurs when the particles aggregate to form larger particles, which is generally the result of small particle size. Ostwald-Mie ripening may occur due to the small particle radius and attendant increase in surface activity, hence solubility. In particular, nanoparticles have a very high surface-to-volume ratio which enhances their dissolution rate and solubility. As a result, the particles may solubilize in the suspension followed by recrystallization to form large crystals. Aggregation and crystal growth result in suspensions of nanoparticles with larger and varying particle sizes. Suspensions with particles larger than 7 μm are no longer suitable for intravenous administration.

[0009] In a suspension, the active ingredient may also undergo degradation and result in reduced activity over time due to interaction with the suspension medium. Even slight dissolution may accelerate the degradation of the active ingredient. The rate of chemical degradation depends on particle size, intrinsic solubility, and the chemical nature of the active ingredient.

[0010] It is highly desirable to have a pharmaceutical preparation of an aqueous suspension with a long shelf life, preferably a minimum of six months in terms of both physical and chemical stabilities.

[0011] Several methods have been described in the prior art to limit aggregation and crystal growth of nanoparticles in suspension to improve their physical stability and shelf-life. One method includes the step of adding surface stabilizers to the preparations. Suitable surface stabilizers include surfactants, polymers, cloud point modifiers (see U.S. Pat. Nos. 5,298,262; 5,346,702; and 5,470,583), crystal growth modifiers (see U.S. Pat. No. 5,665,331), and cryoprotectants (see U.S. Pat. No. 5,302,401). While such approaches have found success in limiting-particle aggregation and crystal growth, suitable surface-active agents may not be found that would enable extended storage of the suspension in the liquid state, either at room temperature or in the refrigerator. Or, if stabilizing agents could be found, they may possess undesirable toxicity profiles.

[0012] Another approach to inhibiting the aggregation and crystal growth of nanoparticles is to limit the average particle size to a narrow range of from about 150 nm to about 350 nm, as described by Liversidge et al. in U.S. Pat. No. 6,267,989. The '989 patent discloses that aggregation and crystal growth are minimized when the particles are within this size range. However, the narrow range of the particle sizes limits its applications. For certain applications, it may be desirable to have nanoparticle suspensions with particle sizes in excess of 400 nm. These applications include, but are not limited to, oral, subcutaneous, or intramuscular administration in which the desirable particle size may be from 5 to 100 microns. In other formulations, the desirable particle size may be smaller than 100 nm. This is true, for example, for particles designed to evade the RES (reticuloendothelial system). Such long-circulating particles can also migrate across loose, fenestrated vasculature such as that associated with certain cancerous tumors. This would facilitate passive targeting of such tumors.

[0013] Yiv et al. discloses in U.S. Pat. No. 6,245,349 a stable formulation of lipid nanoparticles of lipophilic and amphipathic drugs. The formulation is an oil-in-water microemulsion consisting of phospholipid, propylene glycol, polyethylene glycol, a surfactant and water. An oil component such as a triglyceride is optional. The components are blended together to form an emulsion. The average particle size should be smaller than 200 nm-for the preparation to be filter sterilized. The composition can be stored either in a concentrated form or a diluted form. The diluted form includes an aqueous buffer and is stable at a temperature range of about −50° C. to about 40° C. In Example 1, the composition was stored at −20° C. for 21 days with no evidence of phase separation, change in particle size, or drug

crystallization. The method, however, is limited to oil-in-water dispersions with particle sizes smaller than 200 nm, wherein all components are liquids. Such dispersions are commonly sterilized by filter sterilization which requires the dispersion be passed through filters with a pore size of 220 nm.

[0014] The prior art also describes methods of improving the chemical stability of nanoparticle preparations for prolonged storage. The general approach is to remove the aqueous medium by lyophilization and store the nanoparticles in dry, lyophilized form. An example is disclosed in Example 6 of U.S. Pat. No. 5,091,187. Dialysis is generally required before lyophilization to remove any unwanted solutes, such as salt, or to prevent the concentration of such solutes during the lyophilization process. The additional steps of dialysis and lyophilization increase production costs since dialysis is a very time consuming process and lyophilization is an energy consuming process. Furthermore, the lyophilized preparation requires reconstitution with an appropriate dispersing medium before administration either by injection (intravenously, intramuscularly, or subcutaneously), or orally. Such requires more labor in administering the pharmaceutical agent as well as introducing potential human errors that can occur during reconstitution.

[0015] As part of an effort to develop new methods for stabilization of these suspensions, we have discovered that freezing may circumvent these instability mechanisms by encasing the drug particles in a frozen aqueous matrix. At such low temperatures, drug solubility is reduced and very high viscosity of the aqueous medium disfavors diffusion of solute drug away from the solid particle. This includes nucleation, crystal growth and Ostwald ripening. Lower temperatures also increase chemical stability by slowing down drug degradation in the aqueous medium. Crystallization of water may also occur, for example below the eutectic point of the mixture, thus eliminating the possibility of forming a solution phase containing drug which can undergo secondary nucleation, crystal growth and Ostwald ripening.

[0016] The nanoparticles in the invention can be prepared from any of the known methods in the art. One approach centers on reducing the size of the particles that deliver the drug. In one such series of patents, which include U.S. Pat. Nos. 6,228,399; 6,086,376; 5,922,355; and 5,660,858, Parikh et al. discloses that sonication may be used to prepare microparticles of the water-insoluble compound. Of these patents, U.S. Pat. No. 5,922,355 discloses an improvement to a method that uses sonication for making smaller particles. The improvement comprises mixing an active pharmacological agent with a phospholipid and surfactants in a single-phase aqueous system and applying energy to the system to produce smaller particles. Stabilization of the suspension by freezing is not disclosed, however.

[0017] U.S. Pat. No. 5,091,188, issued to Haynes, also discloses reducing the size of particles of a pharmacologically active water-insoluble drug and employing a lipid coating on the particles to confer a solid form. The patent is directed to a pharmaceutical composition consisting essentially of an aqueous suspension of solid particles of the drug having a diameter of about 0.05 to about 10 microns. The lipid coating affixed to the surface of the particles acts to stabilize them. The composition is produced by adding the drug to water in the presence of membrane-forming lipid surfactants and then reducing the particle size within the aqueous suspension. However, freezing the suspension is not disclosed as a stabilization method.

[0018] U.S. Pat. No. 5,858,410 discloses a pharmaceutical nanosuspension suitable for parenteral administration. The '410 patent discloses subjecting at least one solid therapeutically active compound dispersed in a solvent to high pressure homogenization in a piston-gap homogenizer to form particles having an average diameter, determined by photon correlation spectroscopy (PCS) of 10 nm to 1000 nm, the proportion of particles larger than 5 microns in the total population being less than 0.1% (number distribution determined with a Coulter counter), without prior conversion into a melt, wherein the active compound is solid at room temperature and is insoluble, only sparingly soluble or moderately soluble in water, aqueous media and/or organic solvents. The Examples in the '410 patent disclose jet milling prior to homogenization.

[0019] U.S. Pat. No. 5,145,684 discloses another approach to providing nanoparticles of insoluble drugs for parenteral delivery by reducing the size of the particles. The '684 patent discloses the wet milling of an insoluble drug in the presence of a surface modifier to provide a drug particle having an average effective particle size of less than 400 nm. The '684 patent emphasizes the desirability of not using any solvents in its process. The '684 patent discloses the surface modifier is adsorbed on the surface of the drug particle in an amount sufficient to prevent agglomeration into larger particles.

[0020] Besides physically reducing the size of drug particles and coating the particles with a surface stabilizer, nanoparticles can also be prepared by the various methods of precipitation. These methods typically involve dissolving the drug in a solvent as a continuous phase followed by changing the conditions of the solution to a non-continuous phase so that fine particles of the drug precipitate out into the non-continuous phase. A coating agent or surface stabilizer is normally used to co-precipitate with the drug to stabilize the particles. Examples of these precipitation methods are solvent and anti-solvent microprecipitation, phase inversion precipitation, pH shift precipitation, supercritical fluid precipitation, and temperature shift precipitation.

[0021] Examples of appropriate precipitation techniques include preparing nanoparticle suspensions as disclosed in U.S. Patent Application Serial Nos. 60/258,160; 09/874,799; 09/874,637; 09/874,499; and 09/953,979, which are incorporated herein by reference and made a part hereof. These applications disclose forming small particles of organic compounds by dissolving the organic compound in a water miscible organic solvent followed by precipitating the organic compounds in an aqueous medium to form a pre-suspension followed by adding energy to the pre-suspension to stabilize a coating of the particle, to alter the lattice structure of the particle or to reduce particle size. The process is preferably used to prepare a suspension of a poorly water-soluble, pharmaceutically active compound.

[0022] U.S. Pat. No. 5,118,528 discloses a process for preparing nanoparticles by solvent anti-solvent precipitation. The process includes the steps of: (1) preparing a liquid phase of a substance in a solvent or a mixture of solvents to which may be added one or more surfactants, (2) preparing

a second liquid phase of a non-solvent or a mixture of non-solvents, the non-solvent is miscible with the solvent or mixture of solvents for the substance, (3) adding together the solutions of (1) and (2) with stirring; and (4) removing of unwanted solvents to produce a colloidal suspension of nanoparticles. The '528 patent discloses that it produces particles of the substance smaller than 500 nm without the supply of energy. In particular, the '528 patent states that it is undesirable to use high energy equipment such as sonicators and homogenizers.

[0023] U.S. Pat. No. 4,826,689 discloses a method for making uniformly sized particles from water-insoluble drugs or other organic compounds. First, a suitable solid organic compound is dissolved in an organic solvent, and the solution can be diluted with a non-solvent. Then, an aqueous precipitating liquid is infused, precipitating non-aggregated particles with substantially uniform mean diameter. The particles are then separated from the organic solvent. Depending on the organic compound and the desired particle size, the parameters of temperature, ratio of non-solvent to organic solvent, infusion rate, stir rate, and volume can be varied according to the invention. The '689 patent discloses this process forms a drug in a metastable state which is thermodynamically unstable. The '689 patent discloses trapping the drug in a metastable state by utilizing crystallization inhibitors (e.g., polyvinylpyrrolidinone) and surface-active agents (e.g., poly(oxyethylene)-co-(oxypropylene)) to render the metastable precipitate stable enough to be isolated by centrifugation, membrane filtration or reverse osmosis.

[0024] U.S. Pat. No. 5,780,062 discloses a method of preparing small particles of insoluble drugs by (1) dissolving the drug in a water-miscible first solvent, (2) preparing a second solution of a polymer and an amphiphile in an aqueous second solvent in which the drug is substantially insoluble whereby a polymer/amphipbile complex is formed and (3) mixing the solutions from the first and second steps to precipitate an aggregate of the drug and polymer/amphiphile complex.

[0025] U.S. Pat. No. 4,997,454 discloses a method for making uniformly sized particles from solid compounds. The method of the '454 patent includes the steps of dissolving the solid compound in a suitable solvent followed by infusing precipitating liquid thereby precipitating non-aggregated particles with substantially uniform mean diameter. The particles are then separated from the solvent. The '454 patent discourages forming particles in a crystalline state because during the precipitating procedure the crystal can dissolve and recrystallize thereby broadening the particle size distribution range. The '454 patent encourages during the precipitating procedure to trap the particles in a thermodynamically unstable particle state.

[0026] U.S. Pat. Nos. 6,235,224 Bi and 6,143,211, both issued to Mathiowitz et al., disclose the use of phase inversion phenomena to precipitate microencapsulated microparticles. The method includes mixing a polymer and a drug with a solvent. This mixture is introduced into an effective amount of a miscible non-solvent, thereby causing spontaneous formation of the microencapsulated product.

[0027] Microprecipitation by pH shifting is another technology used to prepare dispersions of a nanoparticulate pharmaceutical agent. See, e.g., U.S. Pat. Nos. 5,766,635; 5,716,642; 5,665,331; 5,662,883; 5,560,932; and 4,608,278. This technology involves dissolving a pharmaceutical compound in an aqueous base having a non-neutral pH that is then neutralized to precipitate the compound in the aqueous base.

[0028] In yet another approach, such as that disclosed in U.S. Pat. No. 5,766,635, issued to Spenlenhauer et al., nanoparticles have been prepared by dissolving a poly(ethylene) oxide and/or poly(propylene) oxide/polylactide copolymer in an organic solvent, mixing the organic solution so formed with an aqueous solution to cause nanoparticles to precipitate out of solution, and microfluidizing the suspension without the use of surfactants. Carrier particles consisting of a solid polymer matrix are thus formed, into which a co-precipitated pharmaceutical agent may be incorporated.

[0029] Precipitation by supercritical fluid is disclosed by U.S. Pat. Nos. 5,360,478 and 5,389,263 to Krukonis et al., and WO 97/14407 to Johnston. The technology is similar to the solvent anti-solvent precipitation method. In this case, the supercritical fluid, which can be a gas or liquid at conditions of pressure and temperature above its critical point, acts as the anti-solvent. The addition of the supercritical fluid to a solution of a solute in a solvent causes the solute to attain or approach supersaturated state and to precipitate out as fine particles.

[0030] Temperature shift precipitation is disclosed in U.S. Pat. No. 5,188,837 to Domb. The method involves adding a thermally stable drug to a polymer. The polymer is often oil-based (e.g., phospholipid, synthetic waxes) and has a low melting point. The drug is heated with the polymer to slightly above the melting point of the polymer to form a warm emulsion of the drug in the molten polymer. The emulsion is then cooled quickly by adding the emulsion to a bath of cold non-solvent, such as water, with vigorous shaking to cause the emulsion to form droplets and to solidify to entrap the active agent in a suspension.

[0031] Yet another approach to preparing submicron particles of poorly water soluble organic compounds is the formation of an emulsion of the compound. The organic compound is dissolved in an organic phase. The organic phase forms an emulsion with an aqueous phase. An emulsion evaporation method is disclosed in U.S. patent application Ser. No. 09/964,273. The method includes the steps of: (1) providing a multiphase system having an organic phase and an aqueous phase, the organic phase having a pharmaceutically effective compound therein; and (2) sonicating the system to evaporate a portion of the organic phase to cause precipitation of the compound in the aqueous phase and having an average effective particle size of less than about 400 nm.

[0032] U.S. Pat. No. 5,605,785 discloses a process for forming nanoamorphous dispersions of photographically useful compounds. The process of forming nanoamorphous dispersions include any known process of emulsification that produces a dispersed phase having amorphous particulates.

[0033] Still yet another approach to preparing submicron size nanoparticle suspension of a pharmaceutically active compound is by seeding at some point during a precipitation process to generate crystals of a desired morphology. (see U.S. patent application Ser. No. 10/035,821). The method comprises the steps of dissolving a first quantity of the pharmaceutically-active compound in the water-miscible

first organic solvent to form a first solution. The first solution is then seeded. Alternatively, a second solvent may be seeded. It is also possible to use seed compounds at other points during the precipitation process. The first solution is then mixed with the second solvent. The mixing of the first solution with the second solvent results in the precipitation of the pharmaceutically-active compound in a desired morphological form.

[0034] Another approach is directed to the production of suspended particles coated with protein. U.S. Pat. No. 5,916,596, issued to Desai et al., discloses the application of high shear to a mixture of an organic phase having a pharmacologically active agent dispersed therein and an aqueous medium containing a biocompatible polymer. The mixture is sheared in a high-pressure homogenizer at a pressure in the range of from about 3,000 to 30,000 psi. The '596 patent requires the mixture contain substantially no surfactants because the combined use of a surfactant with a protein results in the formation of large, needle-like crystalline particles that increase in size during storage. See columns 17-18, example 4.

[0035] U.S. Pat. No. 5,560,933, issued to Soon-Shiong et al., discloses the formation of a polymeric shell around the water-insoluble drug for in vivo delivery. The method discloses the application of sonication to a mixture comprising a polymer-containing aqueous medium and a dispersing agent having a substantially water-insoluble drug dispersed therein. In this reference, sonication is used to drive the formation of disulfide bonds in the polymer, causing it to cross-link so as to produce a polymeric shell around the drug. Sonication is conducted for a time sufficient for the disulfide bonds to form.

[0036] In U.S. Pat. No. 5,665,383, Grinstaff et al. discloses the application of ultrasound to a single-phase B i.e., an aqueous medium—to encapsulate an immunostimulating agent within a polymeric shell for in vivo delivery. The ultrasound promotes crosslinking of the encapsulating agent by disulfide bonds to form the shell.

[0037] U.S Pat. Nos. 5,981,719 and 6,268,053 disclose a method of preparing microparticles of macromolecules with particle size of less than 10 microns. Macromolecules are mixed with a soluble polymer or mixture of soluble polymers (e.g., albumin) at a pH near the isoelectric point of the macromolecule in the presence of an energy, preferably heat, for a predetermined length of time. The microparticles formed by this process allow aqueous fluids to enter and solubilized macromolecules and polymers to exit the microparticles and can be made to exhibit short-term or long-term release kinetics, thereby providing either rapid or sustained release of macromolecules.

SUMMARY OF THE INVENTION

[0038] One of the drawbacks of aqueous nanoparticle suspensions is their poor physical and chemical stability. Physical instability is due to particle aggregation and crystal growth. Chemical instability is due to degradation of the active ingredient solubilized in the surrounding solution that is in equilibrium with the suspended solid phase, which can be enhanced due to interactions of active ingredient with excipients such as the surfactants and buffers. Because of these stability problems, many aqueous nanoparticle systems are not suitable for use as pharmaceutical preparations. For example, if the dissolved active compound is chemically unstable due to hydrolysis, for example, then decomposition in solution would shift chemical equilibrium toward progressive degradation and loss of the active ingredient.

[0039] We have discovered that freezing may circumvent these instability mechanisms by encasing the drug particles in a frozen aqueous matrix. At such low temperatures, drug solubility is reduced and very high viscosity of the aqueous medium disfavors diffusion of solute drug away from the solid particle. This includes nucleation, crystal growth and Ostwald ripening. Lower temperatures also slow down the spontaneous degradation of the drug molecules in the aqueous medium to improve their chemical stability. Low temperatures also slow down the degradation of the active ingredient due to its interactions with the excipients. Crystallization of water may also occur, for example below the eutectic point of the mixture, thus eliminating the possibility of forming a solution phase containing drug which can undergo secondary nucleation, crystal growth and Ostwald ripening.

[0040] The present invention provides a composition of a stable nanoparticle suspension of a poorly water soluble pharmaceutical agent in an aqueous matrix and a method for preparing the composition. The present invention contemplates providing a stable suspension of other compounds such as cosmetics, photographically useful agents and the like. The composition can be stored for a prolonged period of time, preferably six months or longer.

[0041] The invention can be applied to any nanoparticle systems known in the art. The nanoparticle suspensions can be prepared from any of the known methods such as physical grinding, homogenization, high shear mixing, emulsion evaporation precipitation, solvent anti-solvent precipitation, supercritical fluid precipitation, temperature shift precipitation, pH shift precipitation, melt precipitation, and seeding.

[0042] The invention is also applicable to nanoparticle systems with a wide range of compositions including, for example, surface modifiers, pH adjusting agents, crystal growth modifiers, cryopreservation agents, osmotic agents, co-solvents and viscosity modulating agents.

[0043] The composition does not require reconstitution with an appropriate dispersing agent before use and is applicable to a variety of routes of administration including, but not limited to, injection (intravenous, intramuscular, subcutaneous), pulmonary, ophthalmic, topical and oral.

[0044] These and other aspects and attributes of the present invention will be discussed with reference to the following drawings and accompanying specification.

DETAILED DESCRIPTION OF THE INVENTION

[0045] While this invention is susceptible of embodiments in many different forms, and will herein be described in detail, preferred embodiments of the invention are disclosed with the understanding that the present disclosure is to be considered as exemplifications of the principles of the invention and are not intended to limit the broad aspects of the invention to the embodiments illustrated.

[0046] The present invention discloses a pharmaceutical composition for intravenous or oral administration of and a

method for preparing the composition as a nanoparticle suspension in an aqueous matrix. Parenteral administration includes intravenous, intra-arterial, intrathecal, intraperitoneal, intraocular, intra-articular, intradural, intramuscular, intradermal or subcutaneous injection. The composition is also suitable for other non-oral routes of administration including, for example, topical, ophthalmic, nasal, buccal, inhalation, rectal, and the like.

[0047] The pharmaceutical agent is preferably a poorly water soluble compound. The composition is physically and chemically unstable when stored in the refrigerator or at room temperature for a prolonged period of time, preferably for one year or longer. Stabilization can be accomplished by freezing the aqueous nanoparticle suspension and storing the composition in the frozen state. At such low temperatures, drug solubility is reduced and the very high viscosity of the aqueous medium disfavors diffusion of a solute drug away from a solid particle containing the drug. This includes nucleation, crystal growth and Ostwald ripening. Lower temperatures also slow down the spontaneous degradation of the drug molecules in the aqueous medium to improve their chemical stability. Crystallization of water may also occur, for example below the eutectic point of the mixture, thus eliminating the possibility of forming a solution phase containing drug which can undergo secondary nucleation, crystal growth and Ostwald ripening.

[0048] The present invention can also be practiced with suspensions of other poorly water soluble materials that are not pharmaceutical agents, including, for example, photographically useful compounds.

[0049] A. Compositions of Nanoparticle Suspensions:

[0050] The composition of the invention comprises nanoparticles of a pharmaceutical agent suspended in a frozen aqueous matrix. One or more excipients can be included in the composition as desired, depending on the particular pharmaceutical agent, the method of preparing the nanoparticle suspension, and the route of administration.

[0051] 1. Pharmaceutical Agents

[0052] The invention can be practiced with a wide variety of pharmaceutical agents which can be a therapeutic agent, a diagnostic agent, or a cosmetic. They include organic and inorganic compounds and biologics such as proteins, peptides, saccharides, polysaccharides, polypeptides, nucleotides, and oligonucleotides.

[0053] The pharmaceutical agent can exist in a crystalline phase or in a non-crystalline, amorphous phase. The agent is preferably poorly water soluble. By "poorly water soluble" it is meant that the pharmaceutical agent has a solubility in water of less than 10 mg/ml, and preferably less than 1 mg/ml. These poorly water soluble agents are most suitable for aqueous nanoparticle suspension preparations since there are limited alternatives of formulating these agents in an aqueous medium.

[0054] The present invention can also be practiced with water soluble pharmaceutical agents, by entrapping these pharmaceutical agents in a solid carrier matrix (for example, polylactate-polyglycolate copolymer, albumin, starch), or by encapsulating these agents in a surrounding vesicle that is impermeable to the pharmaceutical agent. This encapsulating vesicle can be a polymeric coating such as polyacrylate.

Further, the nanoparticles and microparticles prepared from these water soluble pharmaceutical agents can be modified to improve chemical stability and control the pharmacokinetic properties of the agents by controlling the release of the agents from the particles. Examples of water soluble pharmaceutical agents include, but are not limited to, simple organic compounds, proteins, peptides, nucleotides, oligonucleotides, and carbohydrates.

[0055] The therapeutic agent can be selected from a variety of known classes of pharmaceuticals including, for example, analgesics, anti-inflammatory agents, antihelmintics, anti-arrhythmic agents, antibiotics (including penicillins), anticoagulants, antidepressants, antidiabetic agents, antiepileptics, antifungals, antihistamines, antihypertensive agents, antimuscarinic agents, antimycobacterial agents, antineoplastic agents, antiprotozoal agents, immunosuppressants, immunostimulants, antithyroid agents, antiviral agents, anxiolytic sedatives (hypnotics and neuroleptics), astringents, beta-adrenoceptor blocking agents, blood products and substitutes, cardiac inotropic agents, contrast media, corticosteroids, cough suppressants (expectorants and mucolytics), diagnostic agents, diagnostic imaging agents, diuretics, dopaminergics (anti-parkinsonian agents), haemostatics, immunological agents, lipid regulating agents, muscle relaxants, parasympathomimetics, parathyroid calcitonin and biphosphonates, prostaglandins, radio-pharmaceuticals, sex hormones (including steroids), anti-allergic agents, stimulants and anoretics, sympathomimetics, thyroid agents, vasodilators, vaccines and xanthines.

[0056] Diagnostic agents include the x-ray imaging agent and contrast media. Examples of x-ray imaging agents include WIN-8883 (ethyl 3,5-diacetamido-2,4,6-triiodobenzoate) also known as the ethyl ester of diatrazoic acid (EEDA), WIN 67722, i.e., (6-ethoxy-6-oxohexyl-3,5-bis(acetamido)-2,4,6-triiodobenzoate; ethyl-2-(3,5-bis(acetamido)-2,4,6-triiodobenzoyloxy)butyrate (WIN 16318); ethyl diatrizoxyacetate (WIN 12901); ethyl 2-(3,5-bis(acetamido)-2,4,6-triiodobenzoyloxy)propionate (WIN 16923); N-ethyl 2-(3,5-bis(acetamido)-2,4,6-triiodobenzoyloxy acetamide (WIN 65312); isopropyl 2-(3,5-bis(acetamido)-2,4,6-triiodobenzoyloxy)acetamide (WIN 12855); diethyl 2-(3,5-bis(acetamido)-2,4,6-triiodobenzoyloxy malonate (WIN 67721); ethyl 2-(3,5-bis(acetamido)-2,4,6-triiodobenzoyloxy)phenylacetate (WIN 67585); propanedioic acid, [[3,5-bis(acetylamino)-2,4,5-triodobenzoyl]oxy]bis(1-methyl)ester (WIN 68165); and benzoic acid, 3,5-bis(acetylamino)-2,4,6-triodo-4-(ethyl-3-ethoxy-2-butenoate) ester (WIN 68209). Preferred contrast agents include those which are expected to disintegrate relatively rapidly under physiological conditions, thus minimizing any particle associated inflammatory response. Disintegration may result from enzymatic hydrolysis, solubilization of carboxylic acids at physiological pH, or other mechanisms. Thus, poorly soluble iodinated carboxylic acids such as iodipamide, diatrizoic acid, and metrizoic acid, along with hydrolytically labile iodinated species such as WIN 67721, WIN 12901, WIN 68165, and WIN 68209 or others may be preferred.

[0057] Antineoplastic, or anticancer agents, include but are not limited to paclitaxel and derivative compounds, and other antineoplastics selected from the group consisting of alkaloids, antimetabolites, alkylating agents and antibiotics.

[0058] Preferred therapeutic or diagnostic agents include those intended for oral administration and intravenous

administration. A description of these classes of therapeutic agents and diagnostic ageots and a listing of species within each class can be found io Martindale, The Extra Pharmacopoeia, Twenty-ninth Edition, The Pharmaceutical Press, London, 1989 which is incorporated herein by reference and made a part hereof. The therapeutic agents and diagnostic agents are commercially available and/or can be prepared hy techniques koown in the art.

[0059] A cosmetic agent is any active ingredient capable of having a cosmetic activity. Examples of these active ingredients can be, inter alia, emollieots, humectants, free radical-inhibitiog agents, anti-inflammatories, vitamins, depigmenting agents, anti-acne agents, antiseborrhoeics, keratolytics, slimming agents, skio coloriog agents and sunscreen agents, and in particular linoleic acid, retinol, retinoic acid, ascorbic acid alkyl esters, polyunsaturated fatty acids, nicotinic esters, tocopherol nicotinate, uosapooifiables of rice, soybean or shea, ceramides, hydroxy acids such as glycolic acid, seleoium derivatives, antioxidants, beta-carotene, gamma-orizanol and stearyl glycerate. The cosmetics are commercially availahle aod/or can be prepared hy techniques known in the art.

[0060] The pharmaceutical agent can he present in an amount of from about 0.01% to about 50%, more preferably from about 0.1% to ahout 30%, and most preferably from about 0.5% to ahout 5%, hy weight of the composition.

[0061] 2. Excipients

[0062] The excipients in the iovention are optional. Qne or more excipients can be included in the composition. Examples of excipients include huffers, surface modifiers, pH adjusting ageots, crystal growth modifiers, cryopreservation agents, osmotic ageots, co-solveots, and viscosity modulating agents.

[0063] Suitahle surface modifiers cao preferahly he selected from known organic and inorganic pharmaceutical excipients such as ao aoionic surfactant, a cationic surfactant, a nooionic surfactant or a hiological, surface-active molecule.

[0064] Suitable aniooic surfactants include hut are not limited to potassium laurate, sodium lauryl sulfate, sodium dodecylsulfate, alkyl polyoxyethyleoe sulfates, sodium alginate, dioctyl sodium sulfosuccinate, glyceryl esters, sodium carboxymethylcellulose, cholic acid aod other hile acids (e.g., cholic acid, deoxycholic acid, glycocholic acid, taurocholic acid, glycodeoxycholic acid) and salts thereof (e.g., sodium deoxycholate, etc.). Suitable catiooie surfactaots include hut are not limited to quaternary ammonium compounds, such as benzalkonium chloride, cetyltrimethylammonium bromide, lauryldimethylhenzylammonium chloride, acyl carnitine hydmchlorides, or alkyl pyridinium halides.

[0065] Suitable nonionic surfactants include: polyoxyethylene fatty alcohol ethers (Macmgol and Brij), polyoxyethylene sorbitan fatty acid esters (Polysorbates), polyoxyethylene fatty acid esters (Myrj), polyoxyethylene-derivatized lipids or phospholipids, sorbitan esters (Span), glyceml monostearate, polyethylene glycols, polypmpylene glycols, cetyl alcohol, cetostearyl alcohol, stearyl alcohol, aryl alkyl polyether alcohols, polyoxyethylene-polyoxypmpylene copolymers (poloxamers), polaxamines, methylcellulose, hydroxycellulose, hydroxy pmpylcellulose, hydroxy propylmethylcellulose, noncrystalline cellulose, polysaccharides including starch and starch derivaives such as hydroxyethylstarch (HES), polyvinyl alcohol, and polyvinylpyrrolidone. In a preferred form of the invention, the nonionic surfactant is a polyoxyethylene and polyoxypropylene copolymer and preferably a hlock copolymer of propylene glycol and ethylene glycol. Such polymers are sold under the tradename POLOXAMER also sometimes referred to as PLURONIC®, and sold by several suppliers including Spectrum Chemical aod Ruger Among polyoxyethylene fatty acid esters is included those having short alkyl chains Ooe example of such a surfactaot is SOLUTOL® HS 15, polyethylene-660-hydroxystearate, manufactured by BASF Aktiengesellschaft.

[0066] Surface active hiological molecules include such molecules as albumin, casein, heparin, hirudin or other appropriate proteins.

[0067] Other representative examples of surface modifiers include gelatio, casein, gum acacia, cholesterol, tragacanth, stearic acid, benzalkonium chloride, calcium stearate, glycerol monostearate, cetostearyl alcohol, cetomacrogol emulsifying wax, sorbitan esters, polyoxyethylene alkyl ethers, e.g., macrogol ethers such as cetomacrogol 1000, polyoxyethylene castor oil derivatives, polyoxyethylene sorbitan fatty acid esters, e.g., the commercially available Tweens™, polyethylene glycols, polyoxyethylene stearates, colloidal sdicon dioxide, phosphates, sodium dodecylsulfate, carhoxymethylcellulose calcium, carboxymethylcellulose sodium, methylcellulose, hydroxyethylcellulose, hydroxy propylcellulose, hydroxypropylmethylcellulose phtbalate, noncrystalline cellulose, magnesium aluminum silicate, triethanolamine, polyvinyl alcohol, and polyvinylpyrrolidone (PVP). Most of these surface modifiers are known pharmaceutical excipieots aod are described in detail in the Handhook of Pharmaceutical Excipients, puhlished joiotly by the American Pharmaceutical Association and The Pharmaceutical Society of Great Britain, the Pharmaceutical Press, 1986 which is incorporated herein hy reference and made part hereof.

[0068] The surface modifiers are commercially availahle and/or can be prepared hy techniques known in the art. Two or more surface modifiers can he used in combination.

[0069] Suitable pH adjusting agents include but are not limited to buffers, sodium hydroxide, hydrochloric acid, tris(hydroxymethyl)aminomethaoe (tris), citrate, acetate, lactate, meglumine, or the like. Buffers also include but not limited to amino acids such as glycine, leucine, alanine, lysine, or the like.

[0070] Suitable crystal growth modifiers are described in U.S. Pat. No. 5,665,331. A crystal gmwth modifier is defined as a compound that in the co-precipitation process incorporates into the structure of the micmprecipitated crystals of the pharmaceutical agent, thereby hindering growth or enlargement of the microcrystalline precipitate, by the so called Ostwald ripening process. Some crystal growth modifiers may he structurally similar, on a molecular basis, to the pharmaceutical agent. Also suitable as crystal modifiers are polymers such as the crystallization inhibitor polyvinyl pyrrolidinone as disclosed in U.S. Pat. No. 4,826,689. Crystal growth modifiers may also act by forming a complex with the solute that is at supersaturation, and thereby preventing or inhibiting crystal nucleatioo and/or growth.

[0071] Cryoprotectants used in nanoparticle suspensions are disclosed in U.S. Pat. No. 5,302,401. In the '401 patent, cryoprotectants inhibit the agglomeration of nanoparticles during the process of lyophilization. Examples of suitable cryoprotectants include carbohydrates such as sucrose, xylose, glucose, and sugar alcohols such as mannitol and sorbitol, surface active agents such as the polysorbates (Tweens), as well as glycerol and dimethylsulfoxide. Cryoprotectants may also include water-soluble polymers such as polyvinylpyrolidinone (PVP), starch, and polyalkoxy ethers such as polyethylene glycols, polypropylene glycols, and poloxamers. Biologically derived cryoprotectants include albumin. Yet another class of cryoprotectant includes pegylated lipids, such as Solutol. A preferred cryoprotectant is a carbohydrate. A preferred carbohydrate is a monosaccharide or disaccharide. A preferred disaccharide is sucrose. Another preferred cryoprotectant includes polymers such as, but not limited to, those listed above. Yet another preferred cryoprotectant is albumin.

[0072] Viscosity modulating agents are agents that affect the viscosity of the composition. Examples of modulating agents are carbohydrates (e.g. celluloses, gums, sugars, sugar alcohols), polymers (e.g., polnxamers, poloxamines, polyvinylpyrrolidone), proteins (e.g. albumin, milk proteins). These agents are listed in the Handbook of Pharmaceutical Additives published by Gower, under the Section of *Thickeners, Viscosity control agents, Consistency regulators, Bodying agents, Antigellants,* which is incorporated herein by reference and made part hereof.

[0073] Suitable osmotic agents include sugars (e.g. dextrose, sucrose), sugar alcohols (e.g. mannitol, sorbitol), salts (e.g. sodium chloride), glycerol and glycerol derivatives and the like.

[0074] Examples of suitable co-solvents are ethyl alcohol, dimethyl sulfoxide, and N-methyl-2-pyrrolidinone (also called N-methyl-2-pyrrolidone). Other examples include lactic acid, acetic acid and other liquid carboxylic acids.

[0075] The excipient can be present in an amount from about 0.001% tn about 20%, preferably from about 0.01% to about 5%, by weight of the composition.

[0076] The excipient(s) can be added to tbe aqueous medium in the process of preparing of the nanoparticles, or they can he added directly to the pharmaceutical agent before mixing with the aqueous medium. If the pharmaceutical agent is dissolved in an organic phase prior to mixing with an aqueous anti-solvent, the excipient(s) may be added to the organic phase prior to precipitation.

[0077] 3. Particle Size and Shape of Nanoparticles

[0078] In this invention, particle size is measured by dynamic light scattering methods (e.g., photocorrelation spectroscopy, laser diffraction, low-angle laser light scattering (LALLS), medium-angle laser light scattering (MALLS), light obscuration methods (Coulter method, for example), rheology, or microscopy (light or electron) within the ranges set forth above). The invention is applicable to nanoparticle and microparticle suspensions of a wide range of particle sizes. The preferred average effective particle size of the particles is less than about 100 μm, more preferably less than about 7 μm, more preferably less than about 2 μm, and most preferably less than about 400 μm and even more preferably less than about 200 nm or any range or combination of ranges therein.

[0079] 4. Methods of Preparing Nanoparticle Suspensions

[0080] Aqueous nanoparticle suspensions of the pharmaceutical agent can he prepared by any method including mechanical grinding of the active agent, by precipitation tecbniques or hy methods of suspending the pharmaceutical agent. Mechanical grinding include such techniques as jet milling, pearl milling, ball milling, hammer milling, fluid energy milling or wet grinding techniques such as those disclosed in U.S. Pat. No. 5,145,684, which is incorporated herein by reference and made a part hereof.

[0081] The precipitation step can be used to make a particle suspension that is further subjected to an energy-addition step. The energy-addition step includes subjecting tbe particle dispersion to high shear conditions including cavitation, shearing or impact forces utilizing a microfluidizer, piston gap homogenizer or counter-current flow homogenizer such as disclosed in U.S. Pat. No. 5,091,188 which is incorporated herein by reference and made a part hereof. Suitable piston gap homogenizers are commercially available such as those sold under the product name EMULSIFLEX by Avestin, and French Pressure Cells sold by Spectronic Instruments. Suitable microfluidizers are available from Microfluidics Corp. The crystal seeding step described below can be conducted at any point during the process of subjecting the solution to high shear conditions and most preferably is conducted prior to the energy addition step.

[0082] The step of adding energy can also be accomplished using sonication techniques. The step of sonicating can be carried out with any suitable sonication device such as the Branson Model S-450A or Cole-Parmer 500/750 Watt Model. Such devices are well-known in the industry. Typically the sonication device has a sonication horn or probe that is inserted into the drug containing solution to emit sonic energy into the solution. The sonicating device, in a preferred form of tbe invention, is operated at a frequency of from about 1 kHz to about 90 kHz and more preferably from about 20 kHz to about 40 kHz or any range or combination of ranges therein. The probe sizes can vary and preferably is in distinct sizes such as 12 inch or ¼ inch or the like. It may also be desirable to cool the solution during sonication to temperatures below room temperature. The crystal seeding step described helow can be conducted at any point during tbe process of subjecting the solution to high shear conditions and most preferably is conducted before the energy addition step.

[0083] The Method of Precipitation

[0084] In the method of precipitation, the pharmaceutical agent is dissolved in a solvent to derive a solution. The solution is then mixed witb an aqueous medium to derive a pre-suspension of fine particles of the pharmaceutical agent. The aqueous medium may optionally contain one or more excipients selected from the group of surface modifiers, pH adjusting agents, cryoprotective agents, crystal growth modifiers, osmotic agents, co-solvents, and viscosity modifiers. The excipients may also be included in the solvent in wbich the pharmaceutical agent is dissolved, prior to the precipitation step. Energy can be applied to the pre-suspension as needed to stabilize a coating of tlie agent, to change the lattice structure, or to further reduce the size of the particles of the precipitate. Sources of energy include but not limited to sonication, homogenization, microfluidization,

countercurrent homogenization, or other methods of providing impact, shear or cavitation forces. The energy sources also include methods for providing continuous thermal input in the form of heating or cooling, or by temperature variation (e.g., cycling).

[0085] Some known precipitation processes are emulsion evaporation precipitation, microprecipitation, solvent anti-solvent precipitation, supercritical fluid precipitation, temperature shift precipitation, pH shift precipitation, and seeding.

[0086] Emulsion Evaporation Precipitation

[0087] The method of emulsion evaporation is disclosed in U.S. patent application Serial No. 09/964,273, which is incorporated herein by reference and made part hereof. The process comprises the steps of: (1) providing a multiphase system having an organic phase and an aqueous phase, the organic phase having a pharmaceutically effective compound therein; and (2) sonicating the system to evaporate a portion of the organic phase to cause precipitation of the compound in the aqueous phase and having an average effective particle size of less than about 2 μm. The step of providing a multiphase system includes the steps of: (1) mixing a water immiscible solvent (oil phase) with the pharmaceutically effective compound to define an organic solution, (2) preparing an aqueous based solution with one or more surface active compounds, and (3) mixing the organic solution with the aqueous solution to form the multiphase system. The multiphase system can be agitated or mixed to form a crude emulsion. The crude emulsion will have oil droplets in the water of a size of approximately less than about 1 μm in diameter. The crude emulsion is sonicated to define a microemulsion and eventually to define a submicron particle suspension.

[0088] The water immiscible solvent is selected from the group consisting of: linear, branched or cyclic alkanes with carbon number of 5 or higher, linear, branched or cyclic alkenes with carbon number of 5 or higher, linear, branched or cyclic alkynes with carbon number of 5 or higher; aromatic hydrocarbons completely or partially halogenated hydrocarbons, ethers, esters, ketones, mono-, di- or triglycerides, native oils, alcohols, aldehydes, acids, amines, linear or cyclic silicones, hexamethyldisiloxane, or any combination of these solvents. A preferred water immiscible solvent is methylene chloride.

[0089] The sonicating step can be replaced by any other means of providing energy and examples of other sources of energy are sonication, homogenization, microfluidization, countercurrent homogenization, or other methods of providing impact, shear or cavitation forces.

[0090] Microprecipitation

[0091] The method of microprecipitation is disclosed in U.S. Patent Application Serial No. 60/258,160; Ser. Nos. 09/874,799; 09/874,637; 09/874,499; and 09/953,979. Small particles of organic compounds are formed by precipitating an organic compounds in an aqueous medium to form a pre-suspension followed by adding energy to stabilize a coating of the particle or to alter the lattice structure of the particle. The process is preferably used to prepare a suspension of a poorly water-soluble, pharmaceutically active compound suitable for parenteral or oral administration.

[0092] The process can be subdivided into two categories, Method A and Method B.

[0093] Method A

[0094] In Method A, the organic compound ("drug") is first dissolved in the first solvent to define a first solution. The organic compound can be added from about 0.1% (w/v) to about 50% (w/v) depending on the solubility of the organic compound in the first solvent. Heating of the concentrate from about 30° C. to about 100° C. may be necessary to ensure total dissolution of the compound in the first solvent.

[0095] A second aqueous solution is provided with one or more optional surface modifiers such as an anionic surfactant, a cationic surfactant, a nonionic surfactant or a biological surface active molecule added thereto.

[0096] It may also be desirable to add a pH adjusting agent to the second solution such as buffers, sodium hydroxide, hydrochloric acid, tris buffer, citrate, acetate, lactate, meglumine, or the like. Other buffers include amino acids such as glycine, leucine, alanine, lysine, and the like. The second solution should have a pH within the range of from about 2 to about 11.

[0097] In a preferred form of the invention, the method for preparing submicron sized particles of an organic compound includes the steps of adding the first solution to the second solution. The addition rate is dependent on the batch size, and precipitation kinetics for the organic compound. Typically, for a small-scale laboratory process (preparation of 1 liter), the addition rate is from about 0.05 cc per minute to about 10 cc per minute. During the addition, the solutions should be under constant agitation. It has been observed using light microscopy that amorphous particles, semi-crystalline solids, or a supercooled liquid are formed to define a pre-suspension. The method further includes the step of subjecting the pre-suspension to an annealing step to convert the amorphous particles, supercooled liquid or semi-crystalline solid to a crystalline more stable solid state. The resulting particles will have an average effective particle size as measured by dynamic light scattering methods (e.g., photocorrelation spectroscopy, laser diffraction, low-angle laser light scattering (LALLS), medium-angle laser light scattering (MALLS), light obscuration methods (Coulter method, for example), rheology, or microscopy (light or electron) within the ranges set forth above).

[0098] The energy-addition step involves adding energy through sonication, homogenization, countercurrent flow homogenization, microfluidization, or other methods of providing impact, shear or cavitation forces. The sample may be cooled or heated during this stage. In one preferred form of the invention the annealing step is effected by a piston gap homogenizer such as the one sold by Avestin Inc. under the product designation EmulsiFlex-C160. In another preferred form of the invention the annealing may be accomplished by ultrasonication using an ultrasonic processor such as the Vibra-Cell Ultrasonic Processor (600W), manufactured by Sonics and Materials, Inc. In yet another preferred form of the invention, the annealing may be accomplished by use of an emulsification apparatus as described in U.S. Pat. No. 5,720,551 which is incorporated herein by reference and made a part hereof.

[0099] Depending upon the rate of annealing, it may be desirable to adjust the temperature of the processed sample

to within the range of from approximately −30° C. to 30° C. Alternatively, in order to effect a desired phase change in the processed solid, it may also be necessary to heat the pre-suspension to a temperature within the range of from about 30° C. to about 100° C. during the annealing step.

[0100] In addition to amorphous particles, semi-crystalline solids, or a supercooled liquid, the pre-suspension may also consist of friable crystals that are more easily comminuted than in their solid state prior to precipitation. In this case, the energy-addition step breaks down these particles to a desired size.

[0101] Method B

[0102] Method B differs from Method A in the following respects. The first difference is a surfactant or combination of surfactants is added to the first solution. The surfactants may be selected from the groups of nonionic, anionic, and cationic surfactants.

[0103] In addition to amorphous particles, semi-crystalline solids, or a supercooled liquid, the pre-suspension may also consist of friable crystals that are more easily comminuted than in their solid state prior to precipitation. In this case, the energy-addition step breaks down these particles to a desired size.

[0104] One suitable emulsion precipitation technique is disclosed in the co-pending and commonly assigned U.S. Ser. No. 09/964,273, which is incorporated herein by reference and is made a part hereof. In this approach, the process includes the steps of: (1) providing a multiphase system having an organic phase and an aqueous phase, the organic phase having a pharmaceutically effective compound therein; and (2) sonicating the system to evaporate a portion of the organic phase to cause precipitation of the compound in the aqueous phase and having an average effective particle size of less than about 2 μm. The step of providing a multiphase system includes the steps of: (1) mixing a water immiscible solvent with the pharmaceutically effective compound to define an organic solution, (2) preparing an aqueous based solution with one or more surface active compounds, and (3) mixing the organic solution with the aqueous solution to form the multiphase system. The step of mixing the organic phase and the aqueous phase can include the use of piston gap homogenizers, colloidal mills, high speed stirring equipment, extrusion equipment, manual agitation or shaking equipment, microfluidizer, or other equipment or techniques for providing high shear conditions. The crude emulsion will have oil droplets in the water of a size of approximately less than 1 μm in diameter. The crude emulsion is sonicated to define a microemulsion and eventually to define a submicron sized particle suspension.

[0105] An optional polymorph control step discussed in detail below can be conducted during any of these steps. The polymorph control step can be taken prior to, or after sonicating the system. In a most preferred form of the invention, the polymorph control step is conducted during the sonicating step.

[0106] Another approach to preparing submicron sized particles is disclosed in co-pending and commonly assigned U.S. Ser. No. 10/183,035, which is incorporated herein by reference and made a part hereof. The process includes the steps of: (1) providing a crude dispersion of a multiphase system having an organic phase and an aqueous phase, the organic phase having a pharmaceutical compound therein; (2) providing energy to the crude dispersion to form a fine dispersion; (3) freezing the fine dispersion; and (4) lyophilizing the fine dispersion to obtain submicron sized particles of the pharmaceutical compound. The step of providing a multiphase system includes the steps of: (1) mixing a water immiscible solvent with the pharmaceutically effective compound to define an organic solution; (2) preparing an aqueous based solution with one or more surface active compounds; and (3) mixing the organic solution with the aqueous solution to form the multiphase system. The step of mixing the organic phase and the aqueous phase includes the use of piston gap homogenizers, colloidal mills, high speed stirring equipment, extrusion equipment, manual agitation or shaking equipment, microfluidizer, or other equipment or techniques for providing high shear conditions.

[0107] The polymorph control step discussed in detail below can be conducted during any of these steps. In a most preferred form of the invention, the polymorph control step is conducted at the mixing step (3) of the step of providing a multiphase system.

[0108] Solvent Anti-Solvent Precipitation

[0109] A suitable solvent anti-solvent precipitation technique is disclosed in U.S. Pat. Nos. 5,118,528 and 5,100,591 which are incorporated herein by reference and made a part hereof. The process includes the steps of: (1) preparing a liquid phase of a biologically active substance in a solvent or a mixture of solvents to which may be added one or more surfactants; (2) preparing a second liquid phase of a non-solvent or a mixture of non-solvents, the non-solvent is miscible with the solvent or mixture of solvents for the substance; (3) adding together the solutions of (1) and (2) with stirring; and (4) removing of unwanted solvents to produce a colloidal suspension of nanoparticles. The '528 patent discloses that it produces particles of the substance smaller than 500 nm without the supply of energy.

[0110] As above, an optional polymorph control step discussed in detail below can be conducted during any of these steps. In a most preferred form of the invention, the polymorph control step is conducted at step (3) prior to adding together the solutions (1) and (2).

[0111] Phase Inversion Precipitation

[0112] One suitable phase inversion precipitation is disclosed in U.S. Pat. Nos. 6,235,224, 6,143,211 and U.S. Patent Application No. 2001/0042932 which are incorporated herein by reference and made a part hereof. Phase inversion is a term used to describe the physical phenomena by which a polymer dissolved in a continuous phase solvent system inverts into a solid macromolecular network in which the polymer is the continuous phase. One method to induce phase inversion is by the addition of a non-solvent to the continuous phase. The polymer undergoes a transition from a single phase to an unstable two phase mixture: polymer rich and polymer poor fractions. Micellar droplets of non-solvent in the polymer rich phase serve as nucleation sites and become coated with polymer. The '224 patent discloses that phase inversion of polymer solutions under certain conditions can bring about spontaneous formation of discrete microparticles, including nanoparticles. The '224 patent discloses dissolving or dispersing a polymer in a

solvent. A pharmaceutical agent is also dissolved or dispersed in the solvent. For an optional polymorph control step to be effective in this process it is desirable the agent is dissolved in the solvent. The polymer, the agent and the solvent together form a mixture having a continuous phase, wherein the solvent is the continuous phase. The mixture is then introduced into at least tenfold excess of a miscible non-solvent to cause the spontaneous formation of the microencapsulated microparticles of the agent having an average particle size of between 10 nm and 10 μm. The particle size is influenced by the solvent:non-solvent volume ratio, polymer concentration, the viscosity of the polymer-solvent solution, the molecular weight of the polymer, and the characteristics of the solvent-non-solvent pair. The process eliminates the step of creating microdroplets, such as by forming an emulsion, of the solvent. The process also avoids the agitation and/or shear forces.

[0113] The optional polymorph control step discussed in detail below can be conducted during any of these steps. In a most preferred form of the invention, the polymorph control step is conducted prior to or during the adding of the non-solvent to the continuous phase.

[0114] pH Shift Precipitation

[0115] pH shift precipitation techniques typically include a step of dissolving a drug in a solution having a pH where the drug is soluble, followed by the step of changing the pH to a point where the drug is no longer soluble. The pH can be acidic or basic, depending on the particular pharmaceutical compound. The solution is then neutralized to form a pre-suspension of submicron sized particles of the pharmaceutically active compound. One suitable pH shifting precipitation process is disclosed in U.S. Pat. No. 5,665,331, which is incorporated herein by reference and made a part hereof. The process includes the step of dissolving of the pharmaceutical agent together with a crystal growth modifier (CGM) in an alkaline solution and then neutralizing the solution with an acid in the presence of suitable surface-modifying surface-active agent or agents to form a fine particle dispersion of the pharmaceutical agent. The precipitation step can be followed by steps of diafiltration clean-up of the dispersion and then adjusting the concentration of the dispersion to a desired level. This process of reportedly leads to microcrystalline particles of Z-average diameters smaller than 400 nm as measured by photon correlation spectroscopy.

[0116] The optional polymorph control step discussed in detail below can be conducted during any of these steps. In a preferred form of the invention, the polymorph control step is conducted prior to or during the neutralizing step.

[0117] Other examples of pH shifting precipitation methods are disclosed in U.S. Pat. Nos. 5,716,642; 5,662,883; 5,560,932; and 4,608,278, which are incorporated herein by reference and are made a part hereof.

[0118] Infusion Precipitation Method

[0119] Suitable infusion precipitation techniques are disclosed in the U.S. Pat. Nos. 4,997,454 and 4,826,689, which are incorporated herein by reference and made a part hereof. First, a suitable solid compound is dissolved in a suitable organic solvent to form a solvent mixture. Then, a precipitating non-solvent miscible with the organic solvent is infused into the solvent mixture at a temperature between about −10° C. and about 100° C. and at an infusion rate of from about 0.01 ml per minute to about 1000 ml per minute per volume of 50 ml to produce a suspension of precipitated non-aggregated solid particles of the compound with a substantially uniform mean diameter of less than 10 μm. Agitation (e.g., by stirring) of the solution being infused with the precipitating non-solvent is preferred. The non-solvent may contain a surfactant to stabilize the particles against aggregation. The particles are then separated from the solvent. Depending on the solid compound and the desired particle size, the parameters of temperature, ratio of non-solvent to solvent, infusion rate, stir rate, and volume can be varied according to the invention. The particle size is proportional to the ratio of non-solvent:solvent volumes and the temperature of infusion and is inversely proportional to the infusion rate and the stirring rate. The precipitating non-solvent may be aqueous or non-aqueous, depending upon the relative solubility of the compound and the desired suspending vehicle.

[0120] The optional polymorph control step discussed in detail below can be conducted during any of these steps. In a preferred form of the invention, the polymorph control step is conducted prior to or during the infusion of the non-solvent.

[0121] Temperature Shift Precipitation

[0122] Temperature shift precipitation technique, also known as the hot-melt technique, is disclosed in U.S. Pat. No. 5,188,837 to Domb, which is incorporated herein by reference and made a part hereof. In an embodiment of the invention, lipospheres are prepared by the steps of: (1) melting or dissolving a substance such as a drug to be delivered in a molten vehicle to form a liquid of the substance to be delivered; (2) adding a phospholipid along with an aqueous medium to the melted substance or vehicle at a temperature higher than the melting temperature of the substance or vehicle; (3) mixing the suspension at a temperature above the melting temperature of the vehicle until a homogenous fine preparation is obtained; and then (4) rapidly cooling the preparation to room temperature or below.

[0123] The optional polymorph control step discussed in detail below can be conducted during any of these steps provided that the processing temperatures do not exceed the melting point of the drug. In a most preferred form of the invention, the polymorph control step is conducted before the step of cooling the warm drug dispersion.

[0124] Solvent Evaporation Precipitation

[0125] Solvent evaporation precipitation techniques are disclosed in U.S. Pat. No. 4,973,465 which is incorporated herein by reference and made a part hereof. The '465 patent discloses methods for preparing microcrystals including the steps of: (1) providing a solution of a pharmaceutical composition and a phospholipid dissolved in a common organic solvent or combination of solvents, (2) evaporating the solvent or solvents and (3) suspending the film obtained by evaporation of the solvent or solvents in an aqueous solution by vigorous stirring. The solvent can be removed by adding energy to the solution to evaporate a sufficient quantity of the solvent to cause precipitation of the compound. The solvent can also be removed by other well known techniques such as applying a vacuum to the solution

or blowing nitrogen over the solution. The optional polymorph control step discussed in detail below can be conducted during any of these steps. In a most preferred form of the invention, the polymorph control step is conducted prior to the evaporation step.

[0126] Reaction Precipitation

[0127] Reaction precipitation includes the steps of dissolving the pharmaceutical compound into a suitable solvent to form a solution. The compound should be added in an amount at or below the saturation point of the compound in the solvent. The compound is modified by reacting with a chemical agent or by modification in response to adding energy such as heat or UV light or the like to such that the modified compound has a lower solubility in the solvent and precipitates from the solution. The optional polymorph control step discussed in detail below can be conducted during any of these steps. In a most preferred form of the invention, the polymorph control step is conducted prior to or during the precipitation step.

[0128] Compressed Fluid Precipitation

[0129] A suitable technique for precipitating by compressed fluid is disclosed in WO 97/14407 to Johnston, which is incorporated herein by reference and made a part hereof. The method includes the steps of dissolving a water-insoluble drug in a solvent to form a solution. The solution is then sprayed into a compressed fluid, which can be a gas, liquid or supercritical fluid. The addition of the compressed fluid to a solution of a solute in a solvent causes the solute to attain or approach supersaturated state and to precipitate out as fine particles. In this case, the compressed fluid acts as an anti-solvent which lowers the cohesive energy density of the solvent in which the drug is dissolved.

[0130] Alternatively, the drug can be dissolved in the compressed fluid which is then sprayed into an aqueous phase. The rapid expansion of the compressed fluid reduces the solvent power of the fluid, which in turn causes the solute to precipitate out as fine particles in the aqueous phase. In this case, the compressed fluid acts as a solvent.

[0131] In order to stabilize the particles against aggregation, a surface modifier, such as a surfactant, is included in this technique. Particles prepared by this technique are generally 500 nm or smaller.

[0132] The optional polymorph control step discussed in detail below can be conducted during any of these steps. In a most preferred form of the invention, the polymorph control step is conducted prior to or during the particle formation step.

[0133] The Method of Suspension

[0134] The other method of preparing aqueous nanoparticle suspensions is the method of suspension. In this method, particles of the pharmaceutical agent are dispersed in an aqueous medium by adding the particles directly into the aqueous medium to derive a pre-suspension. The particles are normally coated with a surface modifier to inhibit the aggregation of the particles. One or more other excipients can be added either to the pharmaceutical agent or to the aqueous medium.

[0135] Energy may be added to the pharmaceutical agent or the pre-suspension to reduce the sizes of the particles of the pharmaceutical agents to the desired particle size. Examples of sources of energy include but not limited to sonication, homogenization, microfluidization, counter current homogenization, or other methods of providing impact, shear or cavitation forces.

[0136] Polymorph Control

[0137] The methods of preparing a suspension can further include the step of crystal seeding to control the crystal structure of the drug. What is meant by the term "crystal structure" is the arrangement and/or conformation of the molecules within the crystal lattice. Compounds that can be crystallized into different crystal structures are said to be polymorphic. Identification of polymorphs is an important step in drug formulation since different polymorphs of the same drug can show differences in solubility, therapeutic activity, bioavailability, and suspension stability. Similarly, different polymorphs of the same excipient can show differences in solubility, compatibility with the drug to be delivered, chemical stability and suspension stability. Accordingly, it is important to control the polymorphic form of the compound for ensuring product purity and batch-to-batch reproducibility.

[0138] The polymorphic form of the compound in the process discussed above can be controlled by the additional step of seeding. Seeding includes using a seed compound or adding energy to form a seed compound. In a preferred form of the invention, the seed compound is the pharmaceutically-active compound in the desired polymorphic form. Alternatively, the seed compound can also be an inert impurity or an organic compound with a structure similar to that of the desired polymorph.

[0139] The seed compound can be precipitated from a drug containing solution of any of the above-described processes. This method includes the steps of adding the pharmaceutically-active compound in sufficient quantity to exceed the solubility of the pharmaceutically-active compound in the first solution to create a supersaturated solution. The supersaturated solution is treated to precipitate the pharmaceutically-active compound in the desired polymorphic form. Treating the supersaturated solution includes aging the solution for a time period until the formation of a crystal or crystals is observed to create a seeding mixture. Treating the solution also includes subjecting the solution to temperature shifting or pH shifting. It is also possible to add energy to the supersaturated solution to cause the pharmaceutically-active compound to precipitate out of the solution in the desired polymorph. The energy can be added in a variety of ways including the energy addition steps described above. Further energy can be added by heating or exposing the pre-suspension to electromagnetic energy, particle beam or electron beam sources. The electromagnetic energy includes using a laser beam, dynamic electromagnetic energy, or other radiation sources. It is further contemplated utilizing ultrasound, static electric field and a static magnetic field as the energy addition source.

[0140] In a preferred form of the invention, the method for producing seed crystals from an aged supersaturated solution includes the steps of: (i) adding a quantity of the pharmaceutically-active compound to a drug solution to create a supersaturated solution, (ii) aging the supersaturated solution to form detectable crystals to create a seeding mixture; and (iii) precipitating the seeding mixture to create

a pre-suspensioo. The pre-suspensioo can theo be further processed as described herein to provide an aqueous suspension of the pharmaceutically-active compound in the desired polymorph and in the desired size range.

[0141] Seeding can also he accomplished by addiog energy to the first solution or the pre-suspension to form seed compound provided that the exposed liquid or liquids contain the pharmaceutical compouod or a seed material. The energy can be added in the same fashioo as described above for the supersaturated solutioo.

[0142] Accordiogly, the present invention provides a composition of matter of a pharmaceutical compound in a desired polymorphic form essentially free of tbe unspecified polymorph or polymorphs. It is contemplated the methods of this invention can apply used to selectively produce a desired polymorph for numerous pharmaceutical compounds.

[0143] 6. Sterilization of the Composition

[0144] The composition can be heat sterilized or filtered then aseptic processed before freezing, depending on the thermal stability of the particular componeots of the composition and on the particle size of the composition. The preferred method for the production of a sterile product is to filter selected components followed by an aseptic process of manufacture prior to freezing. An alternate method of sterilization for the invention is by gamma irradiation hefore or after the freezing step.

EXAMPLES

Example 1

Preparation of Itraconazole Suspension by use of Microprecipitation Method A with Homogenization Followed by Freezing the Suspension

[0145] Surfactant Solution: To a 4L flask add 3500 mL of distilled water, 22 g of glycerin, 22 g of poloxamer 407, and 22 g of poloxamer 188. The surfactant solution was heated and stirred to dissolve the solids. The surfactant solution was cooled and diluted to 4 liters with distilled water.

[0146] Itraconazole Concentrate: In a 100 mL beaker, 15 g itraconazole and 67.5 g of lactic acid were combined. The mixture was heated to dissolve the solids. The itraconazole concentrate was cooled to room temperature.

[0147] Pre-suspension: The itraconazole concentrate was transferred to a 60 mL syringe. 1.5 liters of surfactant solution was transferred to a jacketed homogenizer bopper. An overhead stirrer was positioned into the diluent solution until the mixing blades were fully immersed. Using a syringe pump, the itraconazole concentrate was added slowly to the diluent solurion with mixing.

[0148] Homogenized suspension: The pre-suspension was immediately homogenized (10,000 psi) for approximately 20 minutes.

[0149] Final suspensioo: The excess lactic acid was removed by centrifuging the homogenized suspension for 20 minutes. The supernatant was discarded and the solids were re-suspeoded in a surfactant solutioo consisting of fresh surfactant solution. The suspensioo was mixed then centrifuged for 20 minutes. The supernatant was discarded and the solids were re-suspended io a surfactant solutioo consisting of fresh surfactant solution. The re-suspended sample was homogenized for approximately 20 mioutes at 10,000 psi. The final pH of the suspension was approximately 4. The suspeosion was collected into 50 mL bottles and sealed with Teflon® faced stoppers.

[0150] Frozeo suspension: 3-50 mL samples of the final suspension was put into a −20° C. freezer and 3-50 mL samples of the final suspension were stored at 2-8° C. After approximately 1 mooth the samples were removed from −20° C. storage and allowed to thaw under ambient conditioos. The samples were transferred to 2-8° C. No phase separatioo, visible aggregation or caking was observed. The sample, which was subjected to freeziog and the controls, wbicb was stored at 2-8° C. and were tested for particle size distribution by laser light scattering. There were no discernable differences in the particle size distribution between the frozen samples and the controls (see below).

Sample ID	Mean Particle Size	99% Particle Size
Control-1	0.243	0.510
Control-1 1 min sonication	0.238	0.510
Control-2	0.240	0.510
Control-2 1 min sonication	0.247	0.510
Control-3	0.250	0.510
Control-3 1 min sonication	0.266	0.510
Freeze-1	0.246	0.510
Freeze-1 1 min sonication	0.261	0.510
Freeze-2	0.232	0.510
Freeze-2 1 min sonication	0.245	0.510
Freeze-3	0.236	0.510
Freeze-3 1 min sonication	0.241	0.510

[0151] It is reasonable to project tbat the frozen suspension to be stable for one year or longer under tbese storage conditions.

Example 2

Amorphous Itraconazole Nanosuspensions are Stabilized by Storage at −70° C.

[0152] Itraconazole (4.0 grams) was dissolved in 20 mL of methylene cbloride and combined with 400 mL of 5% albumin solution (diluted from 25%). The combined solutions were manually shaken to effect dispersion of the two liquids. The crude emulsion was than sonicated (T=5° C.), for 6 minutes (sonicatiog every other 30 seconds using a 1" probe at 40% amplitude). The sonicated solution was rotovapped under house vacuum (~100 torr) for about ½ hour, and than under pump vacuum (<20 torr) for about 2 hours. The rotovapped product was analyzed by light scattering detectioo (Horiba) whicb revealed particles haviog a mean diameter of 406 nio. This product was then sent to Galbraith Laboratories, Inc. for GC headspace analysis, wbicb revealed the methylene chloride concentration to be 12.3 ppm. Inspection by visible light microscopy showed the particles to be spherical in shape with no evidence of crystallinity. Additiooally, x-ray powder diffraction analysis oo particles produced hy this method confirmed them to be completely amorphous.

[0153] Approximately 35 mL of the product was stored at −70 degrees Celsius for 32 days. Re-analysis of the suspen-

sion by HORIBA light scattering detection and microscopic examination revealed essentially no change in particle size (mean value of 427 nm). It is reasonable to project that the frozen suspension to be stable for one year or longer under these storage conditions.

Example 3

1% Budesonide in a PEG-Phospholipid Surfactant System

[0154] Ingredients:

[0155] 1% budesonide

[0156] 1.2% mPEG-PSPE, MW 2000

[0157] 2.25% glycerin

[0158] 0.14% sodium phosphate dibasic

[0159] A weighed quantity of mPEG-PSPE (palmitoyl-stearoyl-phosphatidylethanolamine) and a volume of a previously prepared aqueous solution containing 2.25% glycerin and 0.14% sodium phosphate dibasic at pH 8.6 were combined and mixed using a high-shear mixer. Drug material was added and the blend was mixed under high shear to form a pre-suspension. The pre-suspension was homogenized for 30 discrete passes at a pressure of 25,000 psi.

[0160] A portion of the sample was frozen at −20° C. for 24 hours, and then allowed to thaw completely at room temperature.

Particle Size Results (Measured by Laser Diffractometry)

[0161]

Diameter (Volume-Weighted)	Initial (microns)	After Freeze-Thaw (microns)
Mean	0.8472	0.8371
99th percentile	1.688 1.685	

Example 4

1% Nabumetone with Albumin Surfactant Ingredients:

[0162] 5% human albumin

[0163] 1% nabumetone

[0164] A volume of albumin solution and a weighed quantity of the drug material were combined and mixed under high shear to form a pre-suspension. The pre-suspension was homogenized for 30 discrete passes at a pressure of 25,000 psi.

[0165] A portion of the sample was homogenized at −20° C. for 24 hours, and then allowed to thaw completely at room temperature.

Particle Size Results (Measured by Laser Diffractometry)

[0166]

Diameter (Volume-Weighted)	Initial (microns)	After Freeze-Thaw (microns)
Mean	0.7721 0.7940	
99th percentile	1.889	1.936

Example 5

1% Nabumetone with Polyalkoxyether Surfactant and Bile Salt Ingredients

[0167] 2.2% Poloxamer 188

[0168] 0.1% sodium deoxycholate

[0169] 2.2% glycerin

[0170] 1% nabumetone

[0171] A weighed quantity of the drug material and a volume of a solution containing 2.2% Poloxamer 188, 0.1% sodium deoxycholate, and 2.2% glycerin adjusted to pH 8.7 were combined and mixed under high shear to form a pre-suspension. The pre-suspension was homogenized for 20 discrete passes at a pressure of 25,000 psi.

[0172] A portion of the sample was frozen at −20° C. for 24 hours, and then allowed to thaw completely at room temperature.

Particle Size Results (Measured by Laser Diffractometry)

[0173]

Diameter (Volume-Weighted)	Initial (microns)	After Freeze-Thaw (microns)
Mean	1.0498	1.085
99th percentile	2.423	2.484

Example 6

1% Budesonide with PEG-Fatty Acid Ester Ingredients

[0174] 0.125% Solutol

[0175] 2.25% glycerin

[0176] 1% budesonide

[0177] A weighed quantity of the drug material and a volume of a solution containing 0.125% solutol, and 2.25% glycerin adjusted to pH 8.7 were combined and subjected to high shear mixing to form a pre-suspension. The pre-suspension was homogenized for 30 discrete passes at a pressure of 25,000 psi.

[0178] A portion of the sample was frozen at −20° C. for 24 hours, and then allowed to thaw completely at room temperature.

Particle Size Results (Measured by Laser Diffractometry)

[0179]

Diameter (Volume-Weighted)	Initial (microns)	After Freeze-Thaw (microns)
Mean	0.7587	0.7641
99th percentile	1.460	1.480

Example 7

[0180] 1% Vitamin E TPGS (d-alpha tocopheryl polyethylene glycol 1000 succinate)

[0181] 1% Nabumetone

[0182] 2.25% Glycerin

[0183] 0.14% sodium phosphate dibasic

[0184] Combined a weighed quantity of Vitamin E TPGS and a volume of a premade aqueous solution containing 2.25% glycerin and 0.14% sodium phosphate dibasic at pH 8.6. Stirred the mixture by vortex until the Vitamin E TPGS dissolved. Added the drug material and Ultraturraxed the mixture to form a pre-suspension. Homogenized the pre-suspension with an Avestin B3 homogenizer for 30 discrete passes at a pressure of 25 kpsi.

[0185] Froze a portion of the sample at −20° C. for 24 hours, then allowed it to thaw completely at room temperature.

Particle Size Results

[0186]

	Initial	Freeze-Thaw
Unsonicated 99th% ile	2.372 m	2.593 m
Sonicated 99th% ile	2.266 m	2.398 m
Sonicated mean	1.0332 m	1.0333 m

[0187] While specific embodiments have been illustrated and described, numerous modifications come to mind without departing from the spirit of the invention and the scope of the protection is only limited by the scope of the accompanying claims.

We claim:

1. A composition of a suspension of a poorly water soluble compound comprising particles of the compound suspended in a frozen aqueous matrix.

2. The composition according to claim 1, wherein the compound having a solubility in water of less than 10.0 mg/ml.

3. The composition according to claim 1, wherein the compound is selected from the group consisting of a crystalline phase pharmaceutical agent, an amorphous phase pharmaceutical agent, a crystalline phase cosmetic, and an amorphous phase cosmetic.

4. The composition according to claim 1, wherein the pharmaceutical agent is selected from the group consisting of: therapeutic agents and diagnostic agents.

5. The composition according to claim 4, wherein the therapeutic agent is selected from the group consisting of analgesics, anti-inflammatory agents, anthelmintics, anti-arrhythmic agents, antibiotics, anticoagulants, antidepressants, antidiabetic agents, antiepileptics, antifungals, antihistamines, antihypertensive agents, antimuscarinic agents, antimycobacterial agents, antineoplastic agents, antiprotozoal agents, immunosuppressants, immunostimulants, antithyroid agents, antiviral agents, anxiolytic sedatives, astringents, beta-adrenoceptor blocking agents, contrast media, corticosteroids, cough suppressants, diagnostic agents, diagnostic imaging agents, diuretics, dopaminergics, haemostatics, immunological agents, lipid regulating agents, muscle relaxants, parasympathomimetics, parathyroid calcitonin, prostaglandins, radio-pharmaceuticals, sex hormones, antiallergic agents, stimulants, sympathomimetics, thyroid agents, vasodilators, vaccines and xanthines.

6. The composition according to claim 4, wherein the therapeutic agent is selected from the group consisting of itraconazole, nabumetone and budesonide.

7. The composition according to claim 1, wherein the pharmaceutical agent is present in an amount of from about 0.01 to about 50% by weight based on the total weight of the composition.

8. The composition according to claim 1, wherein the particle size of the pharmaceutical agent is about 50 nm to 50 microns.

9. The composition according to claim 1, wherein the mean diameter of the particles of the pharmaceutical agent is about 50 nm to 2 microns.

10. The composition according to claim 1, wherein about over 99% of the particles having particle size of less than about 5 microns.

11. The composition according to claim 1, further comprising one or more excipients selected from the group consisting of: surface modifiers, pH adjusting agents, crystal growth modifiers, cryopreservation agents, osmotic agents, co-solvents, and viscosity modulating agents.

12. The composition according to claim 11, wherein the surface modifier is selected from the group consisting of: anionic surfactants, cationic surfactants, nonionic surfactants and surface active biological modifiers.

13. The composition according to claim 12, wherein the nonionic surfactant is selected from the group consisting of: polyoxyethylene fatty alcohol ethers, polyoxyethylene sorbitan fatty acid esters, polyoxyethylene fatty acid esters, polyoxyethylene-derivatized lipids such as mPEG-PSPC (palmitoyl-stearoyl-phophatidylcholine), mPEG-PSPE (palmitoyl-stearoyl-phophatidylethanolamine), sorbitan esters, glycerol monostearate, polyethylene glycols, polypropylene glycols, cetyl alcohol, cetostearyl alcohol, stearyl alcohol, aryl alkyl polyether alcohols, polyoxyethylene-polyoxypropylene copolymers, polaxamines, methylcellulose, hydroxycellulose, hydroxy propylcellulose, hydroxy propylmethylcellulose, noncrystalline cellulose, polysaccharides, starch, starch derivatives, hydroxyethylstarch, polyvinyl alcohol, and polyvinylpyrrolidone.

14. The composition according to claim 12, wherein the anionic surfactant is selected from the group consisting of: potassium laurate, triethanolamine stearate, sodium lauryl sulfate, sodium dodecylsulfate, alkyl polyoxyethylene sul-

fates, sodium alginate, dioctyl sodium sulfosuccinate, glyceryl esters, sodium carboxymethylcellulose, bile acids and their salts, cholic acid, deoxycholic acid, glycocholic acid, taurocholic acid, glycodeoxycholic acid, and calcium carboxymethylcellulose.

15. The composition according to claim 12, wherein the cationic surfactant is selected from the group consisting of quaternary ammonium compounds, benzalkonium chloride, cetyltrimethylammonium bromide, chitosans and lauryldimethylbenzylammonium chloride.

16. The composition according to claim 12, wherein the surface active biological modifiers are selected from the group consisting of: albumin, casein, heparin, hirudin, or other proteins.

17. The composition according to claim 11, wherein the pH adjusting agent is selected from the group consisting of: buffers, sodium hydroxide, hydrochloric acid, tris, citrate, acetate, lactate, meglumine, amino acids selected from the group consisting of glycine, alanine, leucine, isoleucine, lysine, methionine, tyrosine, phenylalanine, tryptophan, histidine, proline, serine, glutamic acid, aspartic acid, asparagine, glutamine, cysteine, and taurine.

18. The composition according to claim 11, wherein the cryopreservation agent is selected from the group consisting of carbohydrates, glycerol, polyalkoxyethers, PEG-fatty acids and lipids, biologically-based surfactants, and other surface active agents.

19. The composition according to claim 18, wherein the carbohydrate is selected from the group consisting of saccharides, disaccharides, and sugar alcohols.

20. The composition according to claim 19, wherein the disaccharide is sucrose.

21. The composition according to claim 19, wherein the sugar alcohol is mannitol.

22. The composition according to claim 18, wherein the surface active agent is selected from the group consisting of polysorbate (Tweens), glycerol, polyalkoxyethers, PEG-fatty acids, PEG-lipids, albumin, starch, and dimethylsulfoxide.

23. The composition according to claim 11, wherein the viscosity modulating agent is selected from the group consisting of carbohydrates, polymers, and proteins.

24. The composition according to claim 11, wherein the excipient is present in an amount of from about 0.001% to about 20% based on the total weight of the composition.

25. The composition according to claim 11, wherein the excipient is present in an amount of from about 0.01% to about 5% based on the total weight of the composition.

26. The composition according to claim 1, wherein the suspension is stable for at least 6 months.

27. A method for stabilizing a suspension of a poorly water soluble compound in an aqueous matrix comprising the steps of:

providing the suspension in an aqueous matrix; and

freezing the aqueous suspension.

28. The method according to claim 27, wherein the compound having a solubility in water of less than 10.0 mg/ml.

29. The method according to claim 27, wherein the compound is selected from the group consisting of a crystalline phase pharmaceutical agent, an amorphous phase pharmaceutical agent, a crystalline phase cosmetic, and an amorphous phase cosmetic.

30. The method according to claim 27, wherein the pharmaceutical agent is selected from the group consisting of: therapeutic agents and diagnostic agents.

31. The method according to claim 30, wherein the therapeutic agent is selected from the group consisting of antifungals, analgesics, anti-inflammatory agents, antihelmintics, anti-arrhythmic agents, antibiotics, anticoagulants, antidepressants, antidiabetic agents, antiepileptics, antihistamines, antihypertensive agents, antimuscarinic agents, antiprotozoal agents, antimycobacterial agents, antineoplastic agents, immunosuppressants, immunostimulants, antithyroid agents, antiviral agents, anxiolytic sedatives, astringents, beta-adrenoceptor blocking agents, contrast media, corticosteroids, cough suppressants, diagnostic agents, diagnostic imaging agents, diuretics, dopaminergics, haemostatics, immunological agents, lipid regulating agents, muscle relaxants, parasympathomimetics, parathyroid calcitonin, prostaglandins, radio-pharmaceuticals, sex hormones, antiallergic agents, stimulants, sympathomimetics, thyroid agents, vasodilators vaccines, and xanthines.

32. The method according to claim 30, wherein the therapeutic agent is selected from the group consisting of itraconazole, budesonide, and nabumetone.

33. The method according to claim 27, wherein the pharmaceutical agent is present in an amount of from about 0.01 to about 50% by weight based on the total weight of the composition.

34. The method according to claim 27, wherein the particle size of the pharmaceutical agent is about 50 nm to 50 microns.

35. The method according to claim 27, wherein the mean diameter of the particles of the pharmaceutical agent is about 50 nm to 2 microns.

36. The method according to claim 27, wherein about over 99% of the particles having particle size of less than about 5 microns.

37. The method according to claim 27, further comprising the step of sterilizing by filter sterilization before freezing.

38. The method according to claim 27, further comprising the step of sterilizing by heat sterilization before freezing.

39. The method according to claim 27, further comprising the step of sterilization by gamma-irradiation.

40. The method according to claim 27, wherein the step of providing the suspension is a method selected from the group consisting of:

precipitating the pharmaceutical agent in an aqueous medium to derive a pre-suspension; and

suspending the pharmaceutical agent in an aqueous medium to derive a pre-suspension.

41. The method according to claim 40, wherein the step of precipitating is selected from the group consisting of: microprecipitation, emulsion evaporation, solvent anti-solvent precipitation, supercritical fluid precipitation, temperature shift precipitation, pH shift precipitation, and seeding.

42. The method according to claim 40, wherein the step of suspending comprises the steps of adding the pharmaceutical agent to the aqueous medium.

43. The method according to claim 42, further comprising the step of adding energy to the pharmaceutical agent or to the pre-suspension.

44. The method according to claim 43, wherein the step of adding energy to the pharmaceutical agent comprises performing a method selected from the group consisting of

sonication, homogenization, microfluidization, countercurrent homogenization, and methods nf providing impact, shear or cavitation forces, or thermal energy input, either in a continuous fashion, or hy temperature variatinn.

45. The method according to claim 43, wherein the pharmaceutical agent has particles of a first average particle size prior to the energy-addition step and a second average particle size after the energy-addition step wherein the second average particle size is less than the first average particle size.

46. The method according to claim 40, wherein the pre-suspension further comprising one or more excipients selected from the group consisting of: surface modifiers, pH adjusting agents, crystal growth modifiers, cryopreservatinn agents, osmotic agents, co-solvents and viscosity modulating agents.

47. The method according to claim 46, wherein the surface modifier is selected from the group consisting of: anionic surfactants, cationic surfactants, nonionic surfactants and surface active biological modifiers.

48. The method according to claim 45, wherein the nonionic surfactant is selected from the group consisting of: polyoxyethylene fatty alcohol ethers, polyoxyethylene sorbitan fatty acid esters, polyoxyethylene fatty acid esters, polyoxyethylene-derivatized lipids such as mPEG-PSPC (palmitoyl-stearoyl-phophatidylcholine), mPEG-PSPE (palmitoyl-stearoyl-phophatidylethanolamine), sorbitan esters, glycerol monostearate, polyethylene glycols, polypropylene glycols, cetyl alcohol, cetostearyl alcohol, stearyl alcohol, aryl alkyl polyether alcohols, polyoxyethylene-polyoxypropylene copolymers, polaxamines, methylcellulose, hydroxycellulose, hydroxy propylcellulose, hydroxy pmpylmethylcellulose, noncrystalline cellulose, polysaccharides, starch, starch derivatives, hydroxyethylstarch, polyvinyl alcohol, and polyvinylpyrrolidone

49. The method according to claim 47, wherein the anionic surfactant is selected from the group consisting of potassium laurate, triethanolamine stearate, sodium lauryl sulfate, sodium dodecylsulfate, alkyl polyoxyethylene sulfates, sodium alginate, dioctyl sodium sulfosuccinate, glyceryl esters, sodium carboxymethylcellulose, bile acids and their salts, cholic acid, deoxycholic acid, glycocholic acid, taurocholic acid, glycodeoxycholic acid, and calcium carhoxymethylcellulose.

50. The method according to claim 47, wherein the cationic surfactant is selected from the group consisting of quaternary ammonium compounds, benzalkonium chloride, cetyltrimethylammonium hromide, chitosans and lauryldimethylhenzylammonium chloride

51. The method of claim 47, wherein the surface active biological modifiers are selected from the group consisting of: albumin, casein, heparin, hirudin, or other proteins

52. The method according to claim 46, wherein the pH adjusting agent is selected from the group consisting of: sodium hydroxide, huffers, hydrochlnric acid, tris, citrate, acetate, lactate, meglumineand aminn acids selected from the group glycine, alanine, leucine, isoleucine, lysine, methionine, tyrosine, phenylalanine, tryptophan, histidine, proline, serine, glutamic acid, aspartic acid, asparagine, glutamine, cysteine, and taurine.

53. The method according to claim 46, wherein the cryopreservation agent is selected from the group consisting of carbohydrates, glycerol, polyalkoxyethers, PEG-fatty acids and lipids, biologically-based surfactants, and other surface active agents.

54. The method according to claim 53, wherein the carbohydrate is selected from the group consisting of saccharides, disaccharides, and sugar alcohols

55. The methnd according to claim 54, wherein the disaccharide is sucrose.

56. The method according to claim 54, wherein the sugar alcohol is mannitol.

57. The method according to claim 53, wherein the surface active agent is selected from the group consisting of polysorbates (Tweens), glycerol, polyalkoxyethers, PEG-fatty acids, PEG-lipids, alhumin, starch, and dimethylsulfoxide.

58. The method according to claim 46, wherein the viscosity-modulating agent is selected from the group consisting of carbohydrates, polymers, and proteins.

59. The method according to claim 46, wherein the excipient is present in an amount of ahout 0.001% to ahout 20% based on the total weight of the pre-suspension.

60. The method according to claim 46, wherein the excipient is present in an amount of ahout 0.01% to ahout 5% hased on the total weight of the pre-suspension.

61. The method according to claim 43, wherein the step of adding energy to the pre-suspension comprises the step of performing a method selected from the group consisting of sonication, homogenization, microfluidization, countercurrent homogenization, and methods of providing impact, shear or cavitation forces, or thermal energy input, either in a continuous fashion, or by temperature variation.

62. The method according to claim 41, wherein the emulsion evaporation method comprises the steps of:

dissolving the pharmaceutical agent in a volatile water immiscible solvent to form a solution;

combining the solution with an aqueous medium to form an emulsion;

mixing the emulsion to form a microemulsion; and

removing the volatile water immiscihle solvent in the microemulsion to form an aqueous suspension.

63. The method according to claim 62, wherein the aqueous suspension further comprising one or more excipients selected from the group consisting of surface modifiers, pH adjusting agents, crystal growth modifiers, cryopreservation agents, osmotic agents, co-solvents, and viscosity modulating agent.

64. The method according to claim 63, wherein the surface modifier is selected from the group consisting of: anionic surfactants, cationic surfactants, nonionic surfactants and surface active biological modifiers.

65. The method according to claim 64, wherein the nonionic surfactant is selected from the group consisting of: polyoxyethylene fatty alcohol ethers, polyoxyethylene sorbitan fatty acid esters, polyoxyethylene fatty acid esters, polyoxyethylene-derivatized lipids such as mPEG-PSPC (palmitoyl-stearoyl-phophatidylcholine), mPEG-PSPE (palmitoyl-stearoyl-phophatidylethanolamine), sorbitan esters, glycerol monostearate, polyethylene glycols, polypropylene glycols, cetyl alcohol, cetostearyl alcohol, stearyl alcohol, aryl alkyl polyether alcohols, polyoxyethylene-polyoxypropylene copolymers, polaxamines, methylcellulose, hydroxycellulose, hydroxy propylcellulose,

hydroxy propylmethylcellulose, noncrystalline cellulose, polysaccharides, starch, starch derivatives, hydroxyethylstarch, polyvinyl alcohol, and polyvinylpyrrolidone.

66. The method according to claim 64, wherein the anionic surfactant is selected from the group consisting of: potassium laurate, triethanolamine stearate, sodium lauryl sulfate, sodium dodecylsulfate, alkyl polyoxyethylene sulfates, sodium alginate, dioctyl sodium sulfosuccinate, glyceryl esters, sodium carboxymethylcellulose, bile acids and their salts, cholic acid, deoxycholic acid, glycocholic acid, taurocholic acid, glycodeoxycholic acid, and calcium carboxymethylcellulose.

67. The method according to claim 64, wherein the cationic surfactant is selected from the group consisting of quaternary ammonium compounds, benzalkonium chloride, cetyltrimethylammonium bromide, chitosans and lauryldimethylbenzylammonium chloride.

68. The method according to claim 64, wherein the surface active biological modifiers are selected from the group consisting of: albumin, casein, heparin, hirudin, or other proteins

69. The method according to claim 63, wherein the pH adjusting agent is selected from the group consisting of: buffers, sodium hydroxide, hydrochloric acid, tris, citrate, acetate, lactate, meglumine and amino acids selected from the group consisting of glycine, alanine, leucine, isoleucine, lysine, methionine, tyrosine, phenylalanine, tryptophan, histidine, proline, serine, glutamic acid, aspartic acid, asparagine, glutamine, cysteine, and taurine.

70. The method according to claim 63, wherein the cryopreservation agent is selected from the group consisting of carbohydrates, glycerol, polyalkoxyethers, PEG-fatty acids and lipids, biologically-based surfactants, and other surface active agents

71. The method according to claim 70, wherein the carbohydrate is selected from the group consisting of saccharides, disaccharides, and sugar alcohols.

72. The method according to claim 71, wherein the disaccharide is sucrose.

73. The method according to claim 71, wherein the sugar alcohol is mannitol.

74. The method according to claim 70, wherein the surface active agent is selected from the group consisting of polysorbates (Tweens), polyalkoxyethers, PEG-fatty acids, PEG-lipids, albumin, starch, glycerol, and dimethylsulfoxide.

75. The method according to claim 63, wherein the modulating agent is selected from the group consisting of carbohydrates, polymers, and proteins.

76. The method according to claim 63, wherein the excipient is present in an amount of about 0.001% to about 20% based on the total weight of the suspension.

77. The method according to claim 63, wherein the excipient is present in an amount of about 0.01% to about 5% based on the total weight of the suspension.

78. The method according to claim 62, wherein the volatile water immiscible solvent is selected from the group consisting of: linear, branched or cyclic alkanes with carbon number of 5 or higher, linear, branched or cyclic alkenes with carbon number of 5 or higher, linear, branched or cyclic alkynes with carbon number of 5 or higher; aromatic hydrocarbons completely or partially halogenated hydrocarbons, ethers, esters, ketones, mono-, di- or tri-glycerides, native oils, alcohols, aldehydes, acids, amines, linear or cyclic silicones, hexamethyldisiloxane, or any combination of these solvents

79. The method according to claim 62, wherein the volatile water immiscible solvent is methylene chloride

80. The method according to claim 62, further comprises of the step of cooling the emulsion to about 4° C.

81. The method according to claim 62, wherein the step of mixing comprises the step of adding energy by a method selected form the group consisting of sonication, homogenization, microfluidization, counter current homogenization, and methods of providing impact, shear or cavitation forces, thermal input, either continuously or by temperature variation

82. The method according to claim 62, wherein the step of removing the volatile water immiscible solvent is by sonicating.

83. The method according to claim 62, wherein the step of removing the volatile water immiscible solvent is by placing the microemulsion under a high vacuum.

84. The method according to claim 62, wherein the particles of the pharmaceutical agent are generally spherical in shape.

85. The method according to claim 39, wherein the solvent anti-solvent method comprises the steps of:

dissolving the pharmaceutical agent in a water miscible solvent to form a non-aqueous solution; and

combining the non-aqueous solution with an aqueous medium to precipitate the pharmaceutical agent to derive a pre-suspension.

86. The method according to claim 85, further comprising the step of agitating the pre-suspension to form a suspension.

87. The method according to claim 86, wherein the step of agitating comprises the step of adding energy to the pre-suspension.

88. The method according to claim 87, wherein the energy-addition step comprises the step of importing energy to the pre-suspension using a method selected from the group consisting of sonication, homogenization, microfluidization, counter current homogenization, and methods of providing impact, shear or cavitation forces, or input of thermal energy, either continuously or by temperature variation.

89. The method according to claim 85, wherein the aqueous suspension further comprising one or more excipients selected from the group consisting of surface modifiers, pH adjusting agents, crystal growth modifiers, cryopreservation agents, osmotic agents, co-solvents, and viscosity modulating agent.

90. The method according to claim 89, wherein the surface modifier is selected from the group consisting of anionic surfactants, cationic surfactants, nonionic surfactants and surface active biological modifiers.

91. The method according to claim 84, wherein the nonionic surfactant is selected from the group consisting of polyoxyethylene fatty alcohol ethers, polyoxyethylene sorbitan fatty acid esters, polyoxyethylene fatty acid esters, polyoxyethylene-derivatized lipids such as mPEG-PSPC (palmitoyl-stearoyl-phosphatidylcholine), mPEG-PSPE (palmitoyl-stearoyl-phosphatidylethanolamine), sorbitan esters, glycerol monostearate, polyethylene glycols, polypropylene glycols, cetyl alcohol, cetostearyl alcohol, stearyl alcohol, aryl alkyl polyether alcohols, polyoxyeth-

ylene-polyoxypropylene copolymers, polaxamines, methylcellulose, hydroxycellulose, hydroxy propylcellulose, hydroxy propylmethylcellulose, noncrystalline cellulose, polysaccharides, starch, starch derivatives, hydroxyethylstarch, polyvinyl alcohol, and polyvinylpyrrolidone.

92. The method according to claim 90, wherein the anionic surfactant is selected from the group consisting of: potassium laurate, triethanolamine stearate, sodium lauryl sulfate, sodium dodecylsulfate, alkyl polyoxyethylene sulfates, sodium alginate, dioctyl sodium sulfosuccinate, glyceryl esters, sodium carboxymethylcellulose, bile acids and their salts, cholic acid, deoxycholic acid, glycocholic acid, taurocholic acid, glycodeoxycholic acid, and calcium carboxymethylcellulose.

93. The method according to claim 90, wherein the cationic surfactant is selected from the group consisting of quaternary ammonium compounds, benzalkonium chloride, cetyltrimethylammonium bromide, chitosans and lauryldimethylbenzylammonium chloride.

94. The method according to claim 90, wherein the surface active biological modifiers are selected from the group consisting of: albumin, casein, heparin, hirudin, or other proteins

95. The method according to claim 89, wherein the pH adjusting agent is selected from the group consisting of: buffers, sodium hydroxide, hydrochloric acid, tris, citrate, acetate, lactate, meglumine and amino acids selected from the group consisting of glycine, alanine, leucine, isoleucine, lysine, methionine, tyrosine, phenylalanine, tryptopban, histidine, proline, serine, glutamic acid, aspartic acid, asparagine, glutamine, cysteine, and taurine.

96. The method according to claim 89, wherein the cyropreservation agent is selected from the group consisting of carbohydrates, glycerol, polyalkoxyethers, PEG-fatty acids and lipids, and biologically-based surfactants.

97. The method according to claim 96, wherein the carbohydrate is selected from the group consisting of saccharides, disaccharides, and sugar alcohols.

98. The method according to claim 97, wherein the disaccharide is sucrose.

99. The method according to claim 97, wherein the sugar alcohol is mannitol.

100. The method according to claim 96, wherein the surface active agent is selected from the group consisting of polysorbates (Tweens), polyalkoxyethers, PEG-fatty acids, PEG-lipids, albumin, starch, glycerol, and dimethylsulfoxide.

101. The method according to claim 88, wherein the viscosity modulating agent is selected from the group consisting of carbohydrates, polymers, and proteins.

102. The method according to claim 88, wherein the excipient is present in an amount of about 0.001% to about 20% based on the weight of the pre-suspension.

103. The method according to claim 88, wherein the excipient is present is an amount of about 0.01% to about 5% based on the weight of the pre-suspension.

104. A method for administering a suspension of a poorly water soluble pharmaceutical agent or cosmetic in a frozen aqueous matrix to a patient comprising the steps of:

providing the frozen suspension of the pharmaceutical agent or cosmetic;

thawing the frozen suspension; and

administering the thawed suspension to the patient by a route selected from the group consisting of: parenteral injection (intravenous, intra-arterial, intrathecal, intraperitoneal, intraocular, intra-articular, intradural, intramuscular, intradermal or subcutaneous injection), oral, pulmonary, ophthalmic, or topical.

* * * * *

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(54) Title: CYCLOHEXANEDIOL CRYOPROTECTANT COMPOUNDS

(57) Abstract: A method of cryopreserving cells includes bringing the cells into contact with a cryopreservation composition containing at least one cyclohexanediol compound, and subsequently reducing the temperature of the cells to a cryopreservation temperature. The at least one cyclohexanediol compound is preferably the cis or trans forms of 1,3-cyclohexanediol or 1,4-cyclohexanediol, and racemic mixtures thereof. A preferred cryopreservation composition includes the at least one cyclohexanediol compound and at least one additional cryoprotectant compound.

CYCLOHEXANEDIOL CRYOPROTECTANT COMPOUNDS

[0001] This invention was made with government support under grant number Cooperative Agreement Number 70NANB7H3071, awarded by the Department of Commerce. The government has certain rights in the invention.

BACKGROUND OF THE INVENTION

1. Field of Invention

[0002] This invention relates to particular cyclohexanediol molecules and their use as cryoprotectants.

2. Description of Related Art

[0003] Cryobiology may be defined as the study of the effects of temperatures of lower than normal physiologic ranges upon biologic systems. During the past half-century the fundamentals of the science of cryobiology have evolved to the point where low temperatures are now used extensively as a means to protect and preserve biological systems during enforced periods of ischemia and hypoxia. In practice, preservation is achieved using either hypothermia without freezing, or cryopreservation in which the aqueous system sustains a physical phase change with the formation of ice. Survival of cells from the rigors of freezing and thawing in cryopreservation procedures is only attained by using appropriate cryoprotective agents (CPAs) and in general, these techniques are applicable to isolated cells in suspension or small aggregates of cells in simple tissues. More complex tissues and organs having a defined architecture are not easily preserved using conventional cryopreservation techniques, which is principally due to the deleterious effects of ice formation in an organized multicellular tissue. Simply freezing cells or tissues results in dead, nonfunctional materials.

[0004] The modern era of cryobiology really began with the discovery of the cryoprotective properties of glycerol as reported by Polge et al., "Revival of Spermatazoa After Vitrification and Dehydration at Low Temperatures," Nature, 164:666 (1949). Subsequently, Lovelock et al., "Prevention of Freezing Damage to Living Cells by Dimethyl Sulfoxide," Nature, 183:1394 (1959), discovered that dimethyl sulfoxide was also a cryoprotectant, and despite the wide range of

compounds now known to exhibit cryoprotective properties, it is still the most widely used compound to date.

[0005] A review of the principles of cryobiology can be found in Brockbank, Principles of Cryopreserved Venous Transplantation, Chapter 10, "Essentials of Cryobiology" (1995). A basic principle of cryobiology is that the extent of freezing damage depends upon the amount of free water in the system and the ability of that water to crystallize during freezing. Many types of isolated cells and small aggregates of cells can be frozen simply by following published procedures, but obtaining reproducible results for more complex tissues requires an understanding of the major variables involved in tissue cryopreservation. Major variables involved in tissue freezing include (1) freezing-compatible pH buffers, (2) cryoprotectant choice, concentration and administration, (3) cooling protocol, (4) storage temperature, (5) warming protocol and (6) cryoprotectant elution.

[0006] Many cryoprotectants have been discovered. See, for example, Brockbank, supra. Cryoprotectant selection for cryopreservation is usually restricted to those that confer cryoprotection in a variety of biological systems. On occasion, combinations of cryoprotectants may result in additive or synergistic enhancement of cell survival. Comparison of chemicals with cryoprotectant properties reveals no common structural features. These chemicals are usually divided into two classes: (1) intracellular cryoprotectants with low molecular weights that penetrate cells, and (2) extracellular cryoprotectants with relatively high molecular weights (greater than or equal to sucrose (342 daltons)) which do not penetrate cells. Intracellular cryoprotectants, such as glycerol and dimethyl sulfoxide at concentrations from 0.5 to 3 molar, are effective in minimizing cell damage in many slowly frozen biological systems. Extracellular cryoprotective agents such as polyvinylpyrrolidone or hydroxyethyl starch are often more effective at protecting biological systems cooled at rapid rates.

[0007] What is still desired are improved cryoprotectant materials that increase cell viability during cryopreservation.

SUMMARY OF THE INVENTION

[0008] It is therefore one object of the present invention to provide a cryoprotectant material that effectively protects cells during cryopreservation and achieves increased cell viability upon warming from a frozen state.

[0009] It is still a further object of the present invention to provide a cryoprotectant material that is capable of obtaining consistent and reproducible results in cryopreserving cells and tissues.

[0010] It is a still further object of the present invention to provide a cryoprotectant material that is able to work in conjunction with naturally occurring anti-freeze proteins (AFPs) to promote survival of cells after freezing in a cumulative manner.

[0011] These and other objects are achieved by the present invention, which relates to the use of newly discovered cryoprotectant compounds. In particular, the invention relates to the use of cyclohexanediol compounds, specifically the cis or trans forms of 1,3-cyclohexanediol (1,3CHD) and 1,4-cyclohexanediol (1,4CHD), and their racemic mixtures, as cryoprotectants in preserving living cells.

[0012] In the invention, cells to be cryopreserved are protected against the effects of cryopreservation by bringing the cells into contact with a cryopreservation composition containing at least one cyclohexanediol compound, and subsequently reducing the temperature of the cells to the cryopreservation temperature.

[0013] Also in the invention, the cryopreservation composition preferably comprises not only at least one cyclohexanediol compound, but also at least one additional cryoprotectant compound and/or at least one anti-freeze protein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0014] Figure 1 is a flow chart summarizing the cryopreservation procedure utilized in obtaining the results summarized in this application.

[0015] Figures 2-3 are plots of relative cell viability after freezing using CHD compounds in conjunction with conventional cryoprotective agents.

[0016] Figures 4-5 are plots of relative cell viability after freezing using CHD compounds in conjunction with anti-freeze proteins.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

[0017] The inventors have discovered two new compounds exhibiting cryoprotective activity, 1,3-cyclohexanediol (1,3CHD) and 1,4-cyclohexanediol (1,3CHD). The inventors have also discovered that these compounds are able to work in conjunction with naturally occurring anti-freeze proteins (AFPs) to promote survival after freezing in a cumulative manner.

[0018] Cryopreservation, i.e., the preservation of cells by freezing, in the present invention may be effected in any conventional manner. By "freezing" as used herein is meant temperatures below the freezing point of water, i.e., below 0°C. Cryopreservation typically involves freezing cells to temperatures well below freezing, for example to -130°C or less. The cryopreservation temperature should be less than -20°C, more preferably -80°C or less, most preferably -130°C or less.

[0019] The cells to be cryopreserved using the CHD cryoprotectant compounds of the invention may be in suspension, may be attached to a substrate, etc., without limitation.

[0020] In the method of the invention, the cells to be protected during cryopreservation are first brought into contact with a cryopreservation composition. By being brought into contact with the cryopreservation composition is meant that the cells are made to be in contact in some manner with the cryopreservation composition so that during the reduction of temperature to the cryopreservation temperature, the cells are protected by the cryopreservation composition. For example, the cells may be brought into contact with the cryopreservation composition by filling the appropriate wells of a plate to which the cells to be protected are attached, by suspending the cells in a solution of the cryopreservation composition, etc.

[0021] The cells to be cryopreserved should also preferably be in contact with freezing compatible pH buffer comprised most typically of at least a basic salt solution, an energy source (for example, glucose) and a buffer capable of maintaining a neutral pH at cooled temperatures. Well known such materials include, for example, Dulbecco's Modified Eagle Medium (DMEM). This material may also be included as part of the cryopreservation composition.

[0022] The cryopreservation composition of the invention must contain at least one cyclohexanediol (CHD) compound, for example the cis or trans forms of

1,3-cyclohexanediol or 1,4-cyclohexanediol and racemic mixtures thereof. Preferably, the CHD compound is present in the cryopreservation composition in an amount of from, for example, 0.05 to 2.0 M, more preferably from 0.1 M to 1.0 M.

[0023] The cryopreservation composition also preferably includes a solution well suited for organ storage. The solution can include the buffers discussed above. A particularly preferred solution is, for example, EuroCollins Solution comprised of dextrose, potassium phosphate monobasic and dibasic, sodium bicarbonate and potassium chloride.

[0024] In a further embodiment of the invention, the cryopreservation composition contains not only the CHD compound, but also at least one additional cryoprotectant compound. These additional cryoprotectant compounds may include, for example, any of those set forth in Table 10.1 of Brockbank, supra, including, but not limited to, acetamide, agarose, alginate, l-analine, albumin, ammonium acetate, butanediol, chondroitin sulfate, chloroform, choline, dextrans, diethylene glycol, dimethyl acetamide, dimethyl formamide, dimethyl sulfoxide (DMSO), erythritol, ethanol, ethylene glycol, formamide, glucose, glycerol, α-glycerophosphate, glycerol monoacetate, glycine, hydroxyethyl starch, inositol, lactose, magnesium chloride, magnesium sulfate, maltose, mannitol, mannose, methanol, methyl acetamide, methylformamide, methyl ureas, phenol, pluronic polyols, polyethylene glycol, polyvinylpyrrolidone, proline, propylene glycol, pyridine N-oxide, ribose, serine, sodium bromide, sodium chloride, sodium iodide, sodium nitrate, sodium sulfate, sorbitol, sucrose, trehalose, triethylene glycol, trimethylamine acetate, urea, valine, xylose, etc. This additional cryoprotectant compound is preferably present in the cryopreservation composition in an amount of from, for example, 0.1 M to 10.0 M, preferably 0.1 to 2.0 M.

[0025] In a still further embodiment of the invention, the cryopreservation composition includes the CHD compound, with or without an additional cryoprotectant compound, and also includes an anti-freeze protein/peptide (AFP). AFPs also include anti-freeze glycoproteins (AFGPs) and insect anti-freeze, or "thermal hysteresis" proteins, (THPs). Naturally occurring AFPs are believed to be able to bind to the prism face of developing ice crystals, thereby altering their formation. For the fishes and insects in which these proteins occur, it means a depression of their

freezing point so they are able to survive under conditions that would normally cause their body fluids to freeze.

[0026] Any of the well-known AFPs may be used in the present invention in this regard. See, for example, Sicheri and Yang, Nature, 375:427-431, (1995), describing eight such proteins. Most preferably, the AFP may be, for example, AFPI (AFP type I), AFPIII (AFP type III) and/or AFGP.

[0027] The AFPs may be present in the cryopreservation composition in an amount of from, for example, 0.01 to 1 mg/mL, more preferably 0.05 to 0.5 mg/mL, of composition, for each AFP present.

[0028] Once the cells have been contacted with the cryopreservation composition, the cells may then be frozen for cryopreservation. The cryopreservation and subsequent warming of cells may be conducted in any manner, and may utilize any additional materials, well known in the art. Preferred embodiments are described in the following discussion and the Examples set forth below.

[0029] The cooling (freezing) protocol for cryopreservation in the present invention may be any suitable type. Many types of cooling protocols are well known to practitioners in the art. Most typically, the cooling protocol calls for continuous rate cooling from the point of ice nucleation to -80°C, with the rate of cooling depending on the characteristics of the cells/tissues being frozen as understood in the art (again, see Brockbank, supra). The cooling rate may be, for example, -0.1°C to -10°C per minute, more preferably between -1°C to -2°C per minute. Once the cells are cooled to about -80°C by this continuous rate cooling, they can be transferred to liquid nitrogen or the vapor phase of liquid nitrogen for further cooling to the cryopreservation temperature, which is below the glass transition temperature of the freezing solution (again, typically -130°C or less).

[0030] Once cryopreserved, the cells will subsequently be rewarmed for removal of the cryopreserved cells from the cryopreserved state. The warming protocol for taking the cells out of the frozen state may be any type of warming protocol, which are well known to practitioners in the art. Typically, the warming is done in a one-step procedure in which the cryopreserved specimen is placed into a water bath (temperature of about 37-42°C) until complete rewarming is effected. More rapid warming is also known.

[0031] Most preferably, the cryopreserved cells, particularly cryopreserved cells fixed to a substrate, are warmed by way of the methods described in co-pending U.S. Application No. 60/197,670 filed April 17, 2001, entitled "Novel Warming Method of Cryopreserved Specimens," incorporated herein by reference in its entirety. These methods include a two-step warming protocol, with or without the use of a heat sink.

[0032] The cryopreservation composition of the present invention that includes at least one CHD compound is surprisingly able to increase the survival of cryopreserved cells upon freezing in a cumulative manner. The following examples illustrate the surprising utility of the CHD compounds as a cryoprotectant.

EXAMPLES

Example 1

[0033] A primary cell strain called AV5 was used for these experiments. AV5 cells are derived from porcine heart valve leaflets. Hearts were obtained from pigs and the heart valve leaflets were then removed and washed several times with sterile phosphate-buffered saline (PBS). Small pieces (~1 mm^2) were cut and placed into a 24-well microtiter plate coated with 0.2% gelatin. Dulbecco's Modified Eagle's Medium (DMEM) with 10% fetal calf serum (FCS) was added to just cover the bottom of the well and the plate was left at 37°C with 5% CO_2 in air until visible outgrowth occurred.

[0034] Outgrowth was allowed to continue until cells filled the well at which time the cells were removed from the well using trypsin and placed into a small tissue culture flask. Once the flask reached confluency, the cells were again removed with trypsin and stored in 10% dimethyl sulfoxide (DMSO) at -135°C.

[0035] To evaluate the cryoprotective capabilities of the CHD compounds, the protocol of Figure 1 was followed. AV5 cells were plated the day before each experiment at 25,000 cells/well. At the beginning of each experiment, the plate was placed on ice and the cells were exposed to mannitol prior to loading the various cryopreservation compositions.

[0036] All of the cryopreservation compositions were formulated in EuroCollins Solution, consisting of dextrose, potassium phosphate monobasic and dibasic, sodium bicarbonate and potassium chloride. The plates were then cooled at

the rate of -1.0°C/min to -80°C, and then further cooled with liquid nitrogen vapor and stored overnight at -135°C.

[0037] The next day the plate was removed and warmed to ~4°C at which point it was put back on ice. During warming, 150 µl of 0.5 M mannitol in cell culture media was added to the cells. Once back on ice, the cryoprotectant/mannitol mixture was removed. The cells were washed twice with 0.5 M mannitol/media and twice with DMEM (10%FCS).

[0038] Cell viability was then determined using the non-invasive metabolic indicator Alamar Blue (Trek Diagnostics). Alamar Blue is a fluorescent dye that measures the oxidation/reduction reactions within cells, and thus is indicative of the overall viability of the cells after exposure to cryoprotective agents. A volume of 20 µl Alamar Blue was added to cells in 200 µl of DMEM (10%FCS) and the plate was incubated at 37°C for 3 hours. Fluorescence from Alamar Blue was read in a fluorescent microplate reader (Fmax fluorescent microplate reader by Molecular Dynamics) using an excitation wavelength of 544 nm and an emission wavelength of 590 nm.

[0039] The first set of experiments involved using two CPA compositions, either 1 M dimethyl sulfoxide (DMSO) (left bar of graph at each concentration of 1,3CHD in Figure 2 and 1,4CHD in Figure 3) or a combination of DMSO, formamide and propanediol at a final concentration of 1 M (right bar of graph at each concentration of 1,3CHD in Figure 2 and 1,4CHD in Figure 3). Concentrations varying from 0 to 1 M of 1,3CHD and 0 to 1 M 1,4CHD were added to these two separate CPA compositions for additional experiments. Cell viability was assessed using the assay described above.

[0040] The results are graphically summarized in Figures 2 and 3. Figure 2 relates to 1,3CHD, while Figure 3 relates to 1,4CHD. For each, the data was normalized to the conventional cryoprotectant alone and is the mean (+/- SEM) of 12 replicates. As shown in these two Figures, in the presence of varying concentrations of both CHD molecules, viability was significantly increased over the comparative cryopreservation compositions that contained only the conventional cryoprotectants without a CHD compound (i.e., concentration of 0.00 CHD).

[0041] Similar results to the foregoing for AV5 have been obtained with other cell types, including (1) A10, an established cell line of smooth muscle cells derived from rat thoracic aorta and (2) J15, a primary cell strain of smooth muscle cells derived from rabbit jugular veins.

Example 2

[0042] In this Example, an anti-freeze protein (AFP) was added to the cryoprotective composition. Varying concentrations of three different AFPs (AFPI, AFPIII and AFGP) were used along with 1 M DMSO and either 0.25 M 1,3CHD (Figure 5) or 0.5 M 1,4CHD (Figure 4). Again, the protocol of Figure 1 was followed. The data were normalized to the comparative conventional cryoprotectant alone (i.e., DMSO alone), and the results are presented in Figures 4 and 5.

[0043] In Figure 4, the relative cell viability of AV5 cells after freezing using a combination of 1,4CHD/AFPI/DMSO is summarized. Concentrations of the constituents included 0.5 M 1,4CHD, 0.1 mg/mL AFPI and 1 M DMSO. The graph depicts viability in the presence of 1 M DMSO alone or in combination with 1,4CHD or 1,4CHD plus AFPI at the above concentrations. Data was normalized to the conventional cryoprotectant (DMSO) alone and is the mean (+/- SEM) of 3 replicates.

[0044] In Figure 4, the results demonstrated the increased viability of the cells upon addition of the CHD molecule as compared to the conventional cryoprotectant alone and a further increase in viability upon addition of the AFPI protein to the conventional cryoprotectant/CHD mixture. Thus, a cumulative effect in the presence of AFP and CHD was demonstrated.

[0045] In Figure 5, the relative cell viability of AV5 cells after freezing using a combination of 1,3CHD, DMSO and three different AFP proteins is summarized. Concentrations of the constituents included 0.25 M 1,3CHD, 1 M DMSO and 0.1 mg/mL for each AFP protein (left bar of graph is with AFPI, middle bar of graph is with AFPIII, and right bar of graph is with AFGP). Data was normalized to the DMSO alone and is the mean (+/- SEM) of 3 replicates.

[0046] From the data of Figure 5, AFPI appears to confer the best protection to the cells in this Example as observed by an increase in cell viability as compared to the conventional cryoprotectant alone or to the conventional cryoprotectant/CHD

mixture. This cumulative increase in viability is not observed in the presence of the AFPs plus a conventional cryoprotectant alone (i.e., without the CHD present).

[0047] While this invention has been described in conjunction with specific embodiments thereof, it is evident that many alternatives, modifications and variations will be apparent to those skilled in the art. Accordingly, the preferred embodiments of the invention as set forth herein are intended to be illustrative only, and not limiting. Various changes may be made without departing from the spirit and scope of the invention as defined in the following claims.

WHAT IS CLAIMED IS:

1. A method of cryopreserving cells, comprising bringing the cells into contact with a cryopreservation composition containing at least one cyclohexanediol compound, and subsequently reducing the temperature of the cells to a cryopreservation temperature.

2. A method according to claim 1, wherein the at least one cyclohexanediol compound is selected from the group consisting of the cis or trans forms of 1,3-cyclohexanediol and 1,4-cyclohexanediol, and racemic mixtures thereof.

3. A method according to claim 1, wherein the cyclohexanediol compound is present in the cryopreservation composition in an amount of from 0.05 to 2.0 M.

4. A method according to claim 1, wherein the cryopreservation composition further contains at least one additional cryoprotectant compound.

5. A method according to claim 4, wherein the at least one additional cryoprotectant compound is selected from the group consisting of including acetamide, agarose, alginate, l-analine, albumin, ammonium acetate, butanediol, chondroitin sulfate, chloroform, choline, dextrans, diethylene glycol, dimethyl acetamide, dimethyl formamide, dimethyl sulfoxide (DMSO), erythritol, ethanol, ethylene glycol, formamide, glucose, glycerol, α-glycerophosphate, glycerol monoacetate, glycine, hydroxyethyl starch, inositol, lactose, magnesium chloride, magnesium sulfate, maltose, mannitol, mannose, methanol, methyl acetamide, methylformamide, methyl ureas, phenol, pluronic polyols, polyethylene glycol, polyvinylpyrrolidone, proline, propylene glycol, pyridine N-oxide, ribose, serine, sodium bromide, sodium chloride, sodium iodide, sodium nitrate, sodium sulfate, sorbitol, sucrose, trehalose, triethylene glycol, trimethylamine acetate, urea, valine and xylose.

6. A method according to claim 4, wherein the at least one additional cryoprotectant compound is present in the cryopreservation composition in an amount of from 0.1 to 10.0 M.

7. A method according to claim 1, wherein the cryopreservation composition further contains at least one anti-freeze protein.

8. A method according to claim 7, wherein the anti-freeze protein is present in the cryopreservation composition in an amount of from 0.01 to 1 mg/mL of the cryopreservation composition.

9. A method according to claim 4, wherein the cryopreservation composition further contains at least one anti-freeze protein.

10. A method according to claim 1, wherein the cryopreservation temperature is –20°C or less.

11. A cryopreservation composition comprising at least one cyclohexanediol compound and at least one additional cryoprotectant compound.

12. A cryopreservation composition according to claim 11, wherein the at least one cyclohexanediol compound is selected from the group consisting of the cis or trans forms of 1,3-cyclohexanediol and 1,4-cyclohexanediol, and racemic mixtures thereof.

13. A cryopreservation composition according to claim 11, wherein the cyclohexanediol compound is present in the cryopreservation composition in an amount of from 0.05 to 2.0 M.

14. A cryopreservation composition according to claim 11, wherein the at least one additional cryoprotectant compound is selected from the group consisting of acetamide, agarose, alginate, l-analine, albumin, ammonium acetate, butanediol, chondroitin sulfate, chloroform, choline, dextrans, diethylene glycol, dimethyl acetamide, dimethyl formamide, dimethyl sulfoxide (DMSO), erythritol, ethanol, ethylene glycol, formamide, glucose, glycerol, α-glycerophosphate, glycerol monoacetate, glycine, hydroxyethyl starch, inositol, lactose, magnesium chloride, magnesium sulfate, maltose, mannitol, mannose, methanol, methyl acetamide, methylformamide, methyl ureas, phenol, pluronic polyols, polyethylene glycol, polyvinylpyrrolidone, proline, propylene glycol, pyridine N-oxide, ribose, serine, sodium bromide, sodium chloride, sodium iodide, sodium nitrate, sodium sulfate, sorbitol, sucrose, trehalose, triethylene glycol, trimethylamine acetate, urea, valine and xylose.

15. A cryopreservation composition according to claim 11, wherein the at least one additional cryoprotectant compound is present in the cryopreservation composition in an amount of from 0.1 to 10.0 M.

16. A cryopreservation composition according to claim 11, wherein the cryopreservation composition further contains at least one anti-freeze protein.

17. A cryopreservation composition according to claim 16, wherein the anti-freeze protein is present in the cryopreservation composition in an amount of from 0.01 to 1 mg/mL of the cryopreservation composition.

18. A cryopreservation composition according to claim 11, wherein the cryopreservation composition further contains at least one anti-freeze glycoprotein.

19. A cryopreservation composition according to claim 18, wherein the anti-freeze glycoprotein is present in the cryopreservation composition in an amount of from 0.01 to 1 mg/mL of the cryopreservation composition.

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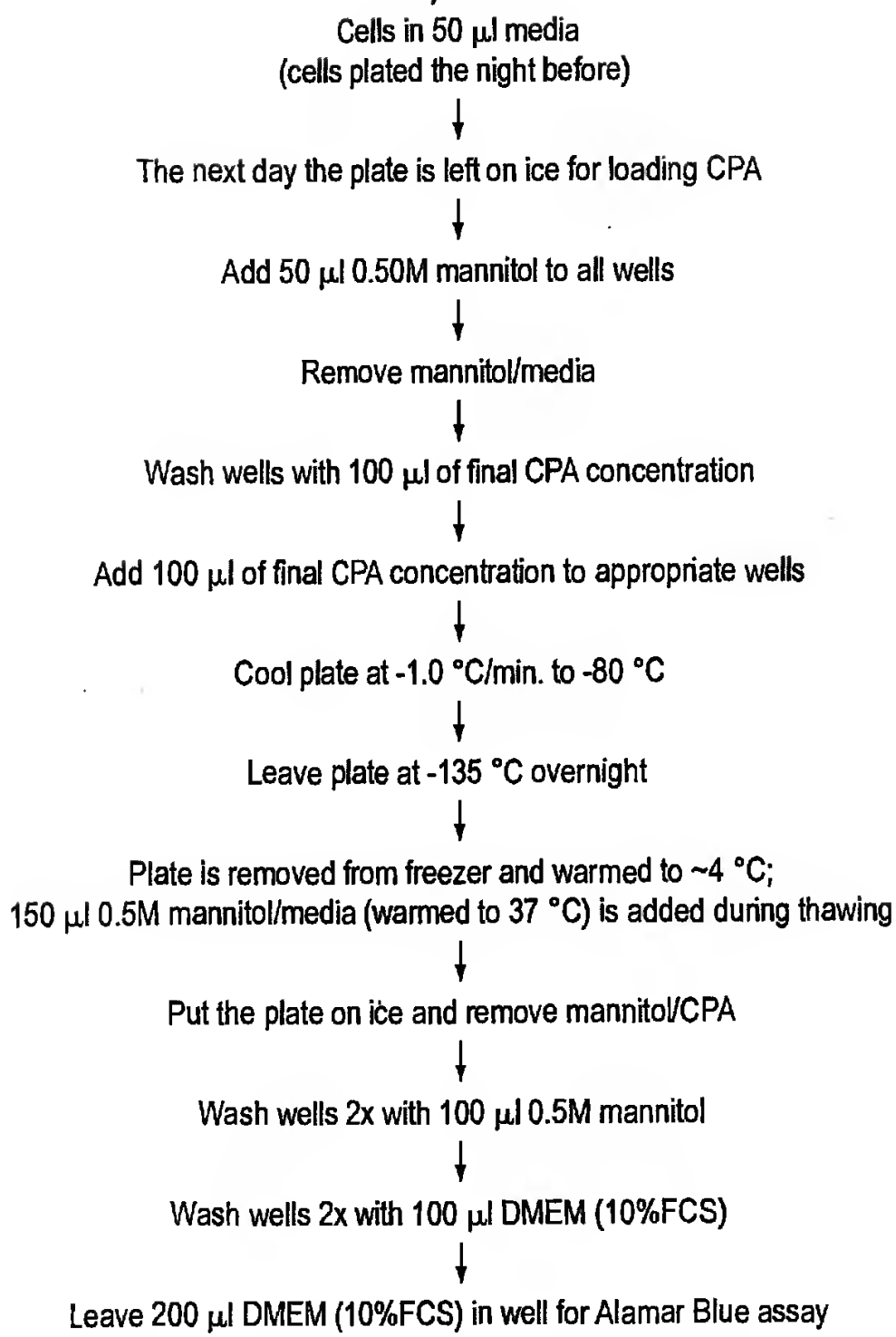
Cells in 50 µl media
(cells plated the night before)
The next day the plate is left on ice for loading CPA
Add 50 µl 0.50M mannitol to all wells
Remove mannitol/media
Wash wells with 100 µl of final CPA concentration
Add 100 µl of final CPA concentration to appropriate wells
Cool plate at -1.0 °C/min. to -80 °C
Leave plate at -135 °C overnight
Plate is removed from freezer and warmed to ~4 °C;
150 µl 0.5M mannitol/media (warmed to 37 °C) is added during thawing
Put the plate on ice and remove mannitol/CPA
Wash wells 2x with 100 µl 0.5M mannitol
Wash wells 2x with 100 µl DMEM (10%FCS)
Leave 200 µl DMEM (10%FCS) in well for Alamar Blue assay

FIG. 1

2/3

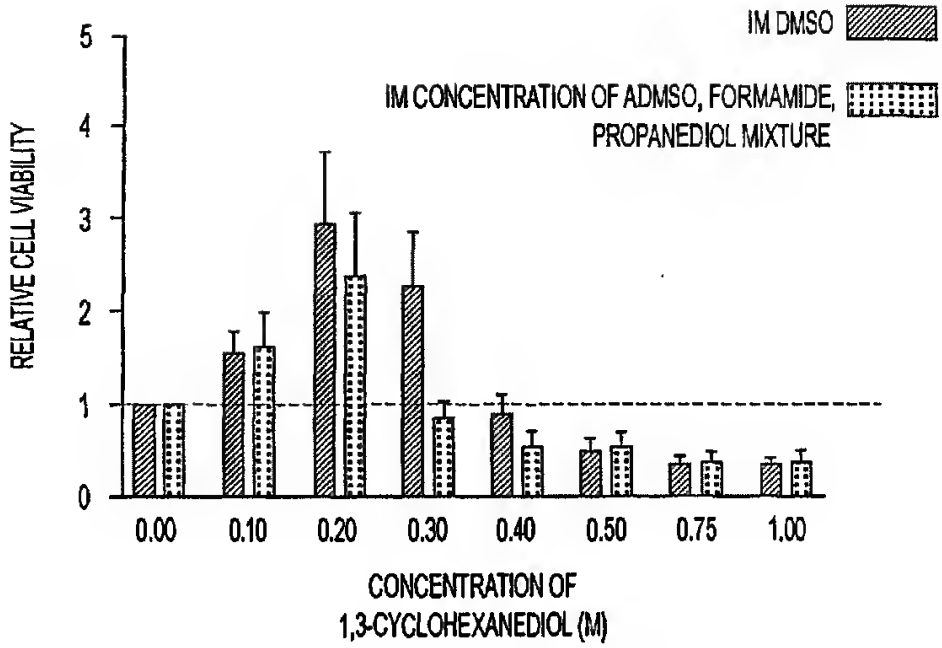
1M DMSO
1M CONCENTRATION OF ADMSO, FORMAMIDE, PROPANEDIOL MIXTURE
RELATIVE CELL VIABILITY
0
1
2
3
4
5
0.00
0.10
0.20
0.30
0.40
0.50
0.75
1.00
CONCENTRATION OF 1,3-CYCLOHEXANEDIOL (M)

FIG. 2

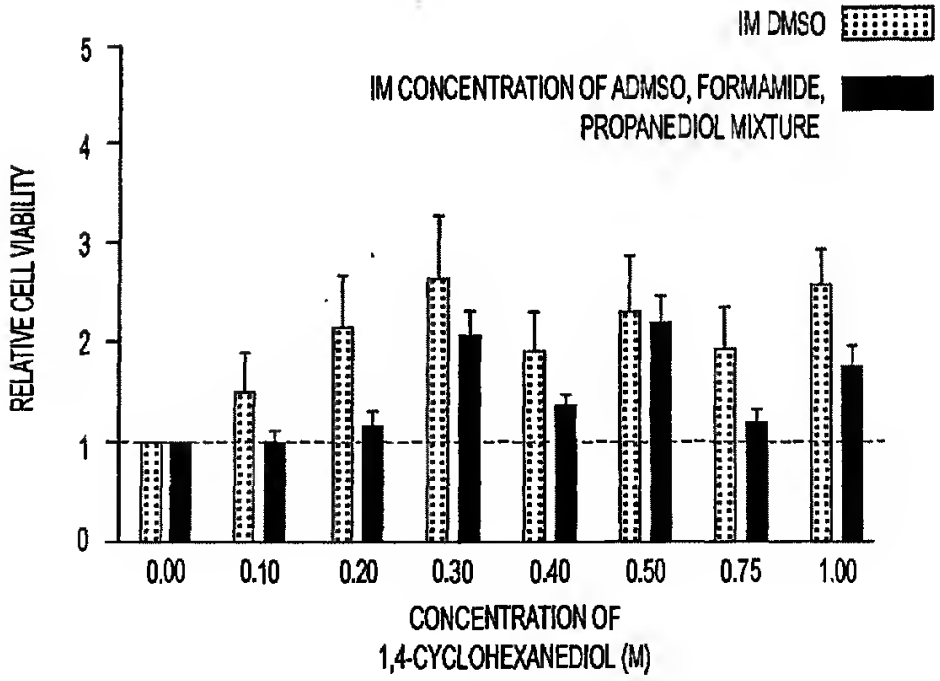
1M DMSO
1M CONCENTRATION OF ADMSO, FORMAMIDE, PROPANEDIOL MIXTURE
RELATIVE CELL VIABILITY
0
1
2
3
4
5
0.00
0.10
0.20
0.30
0.40
0.50
0.75
1.00
CONCENTRATION OF 1,4-CYCLOHEXANEDIOL (M)

FIG. 3

3/3

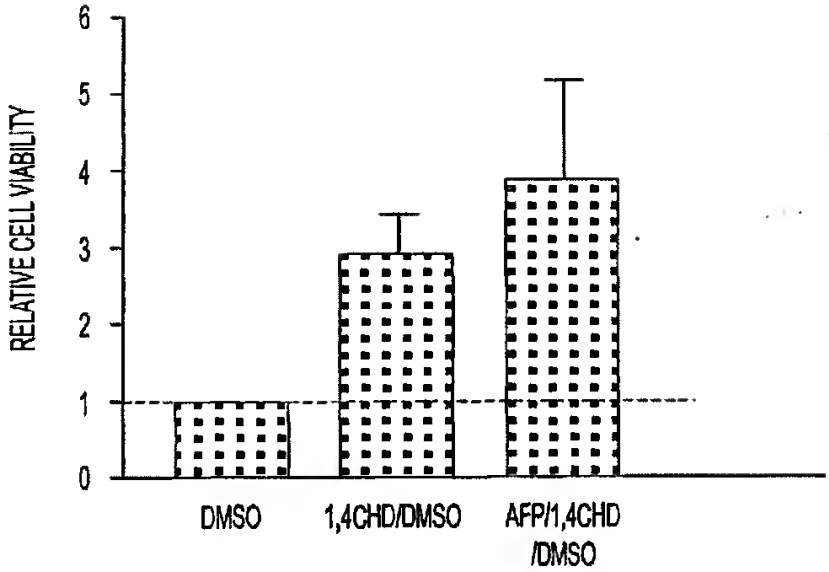
RELATIVE CELL VIABILITY
0
1
2
3
4
5
6
DMSO
1,4CHD/DMSO
AFP/1,4CHD /DMSO

FIG. 4

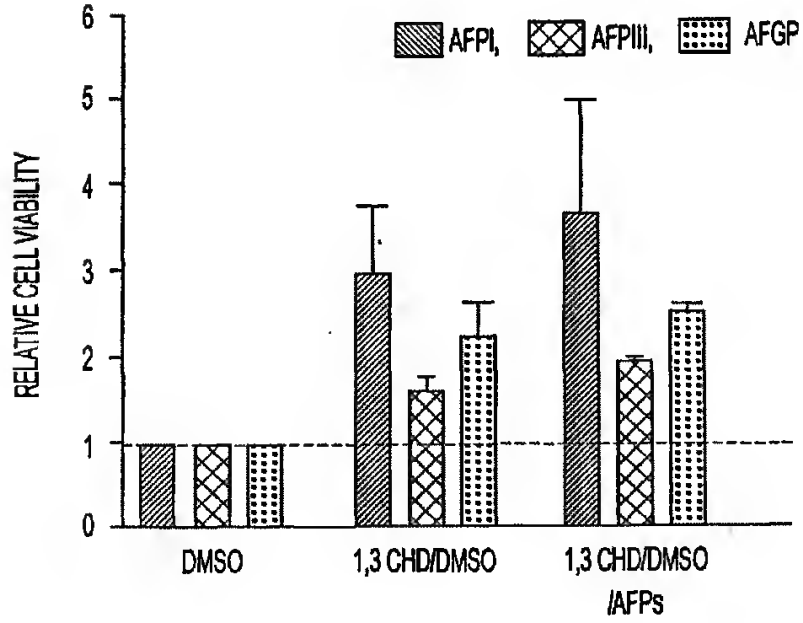
AFPI,
AFPIII,
AFGP
RELATIVE CELL VIABILITY
0
1
2
3
4
5
6
DMSO
1,3 CHD/DMSO
1,3 CHD/DMSO /AFPs

FIG. 5

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 01/12465

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A01N1/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, BIOSIS, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
X	WO 99 18169 A (LIFE SCIENCE HOLDINGS INC ;ORGAN INC (US)) 15 April 1999 (1999-04-15) claims 2,18,19,21 page 18, paragraph 2 page 25, paragraph 3 - paragraph 4 page 27, paragraph 4 - paragraph 5 ---	1-19
X	WO 00 16618 A (21ST CENTURY MEDICINE INC) 30 March 2000 (2000-03-30) page 29, line 5 claims 1,10-20 page 9, line 34-36 --- -/--	1-19

[X] Further documents are listed in the continuation of box C. [X] Patent family members are listed in annex

* Special categories of cited documents
'A' document defining the general state of the art which is not considered to be of particular relevance
'E' earlier document but published on or after the international filing date
'L' document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
'O' document referring to an oral disclosure, use, exhibition or other means
'P' document published prior to the international filing date but later than the priority date claimed
'T' later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
'X' document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
'Y' document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
'&' document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 September 2001	25/09/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax. (+31-70) 340-3016	Decorte, D

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 01/12465

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
X	DATABASE CHEMABS 'Online! CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US; O'CONNELL, KATHLEEN M. ET AL: "Cryoprotectants for Crithidia fasciculata stored at -20.deg.. Trypanosoma gambiense and T. conorhini" retrieved from STN Database accession no. 70:103995 XP002177168 abstract & J. PROTOZOOL. (1968), 15(4), 719-24 , -----	1-19

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 01/12465

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9918169 A	15-04-1999	AU 9784298 A	27-04-1999
		EP 1019458 A	19-07-2000
WO 0016618 A	30-03-2000	AU 1093900 A	10-04-2000
		AU 6499299 A	10-04-2000
		EP 1115281 A	18-07-2001
		WO 0016619 A	30-03-2000

US 20050004049A1

(19) **United States**

(12) **Patent Application Publication** (10) **Pub. No.: US 2005/0004049 A1**

Liversidge (43) **Pub. Date: Jan. 6, 2005**

(54) **NOVEL GRISEOFULVIN COMPOSITIONS**

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(21) Appl. No.: **10/683,154**

(22) Filed: **Oct. 14, 2003**

Related U.S. Application Data

(63) Continuation-in-part of application No. 10/175,851, filed on Jun. 21, 2002, now abandoned, which is a continuation of application No. 08/815,346, filed on Mar. 11, 1997, now Pat. No. 6,432,381.

Publication Classification

(51) **Int. Cl.**[7] **A61K 31/704**

(52) **U.S. Cl.** **514/35**

(57) **ABSTRACT**

The present invention is directed to nanoparticulate compositions comprising griseofulvin. The griseofulvin particles of the composition preferably have an effective average particle size of less than about 2 microns.

NOVEL GRISEOFULVIN COMPOSITIONS

[0001] CROSS-REFERENCE TO RELATED APPLICATIONS

[0002] This application is a continuatinn-in-part of U.S. Application Ser. No. 10/175,851, filed on Jun. 21, 2002 (pending), wbich is a contionation of U.S. Application Ser. No. 08/815,346, filed on Mar. 11, 1997, now U.S. Pat. No. 6,432,381.

FIELD OF THE INVENTION

[0003] The present invention relates to a novel compositions of griseofulvin, comprising griseofulvin particles having an effective average particle size of less than about 2000 nm and at least one surface stabilizer.

BACKGROUND OF THE INVENTION

[0004] 1. Background Regarding Nanoparticulate Active Agent Compositions

[0005] Nanoparticulate active agent cnmpositions, first described in U.S. Pat. No. 5,145,684 ("the '684 patent"), are particles consisting of a poorly soluble therapeutic nr diagnostic agent baving associated with the surface thereof a non-crosslinked surface stabilizer. The '684 patent does not describe nanoparticulate griseofulvin compositions.

[0006] Methods of making nanoparticulate active agent cnmpositions are described, for example, in U.S. Pat. Nos. 5,518,187 and 5,862,999, both for "Method of Grinding Pharmaceutical Substances," U.S. Pat. No. 5,718,388, for "Continuous Method of Grinding Pharmaceutical Substances;" and U.S. Pat. No. 5,510,118 for "Process of Preparing Therapeutic Compositions Containing Nanoparticles." These patents do not describe methods nf making nanoparticulate griseofulvin.

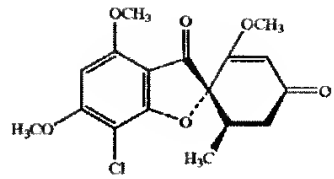
[0007] Nanoparticulate active agent compositions are also described, for example, in U.S. Pat. No. 5,298,262 for "Use of Ionic Cloud Point Modifiers to Prevent Particle Aggregation During Sterilization;" U.S. Pat. No. 5,302,401 fnr "Metbod to Reduce Particle Size Growth During Lyophilization;" U.S. Pat. No. 5,318,767 for "X-Ray Contrast Compositions Useful in Medical Imaging;" U.S. Pat. No. 5,326,552 for "Novel Formulation For Nanoparticulate X-Ray Blood Pool Contrast Agents Using High Molecular Weight Non-ionic Surfactants;" U.S. Pat. No. 5,328,404 for "Method of X-Ray Imaging Using Iodinated Aromatic Propanedioates;" U.S. Pat. No. 5,336,507 for "Use of Charged Phospholipids to Reduce Nanoparticle Aggregatinn;" U.S. Pat. No. 5,340,564 for "Formulations Comprising Olin 10-G to Prevent Particle Aggregation and Increase Stability;" U.S. Pat. No. 5,346,702 for "Use of Non-Ionic Cloud Point Modifiers to Minimize Nanoparticulate Aggregation During Sterilization;" U.S. Pat. No. 5,349,957 for "Preparation and Magnetic Properties of Very Small Magnetic-Dextran Particles;" U.S. Pat. No. 5,352,459 for "Use of Purified Surface Modifiers to Prevent Particle Aggregation During Sterilization;" U.S. Pat. Nos. 5,399,363 and 5,494,683, both for "Surface Modified Anticancer Nanoparticles;" U.S. Pat. No. 5,401,492 for "Water Insoluble Non-Magnetic Manganese Particles as Magnetic Resonance Enhancement Agents;" U.S. Pat. No. 5,429,824 for "Use of Tyloxapol as a Nanoparticulate Stabilizer;" U.S. Pat. No. 5,447,710 for "Method for Making Nanoparticulate X-Ray Blood Pool Contrast Agents Using High Molecular Weight Non-ionic Surfactants;" U.S. Pat. Nn. 5,451,393 for "X-Ray Contrast Compositions Useful in Medical Imaging;" U S Pat. No. 5,466,440 for "Formulations of Oral Gastrointestinal Diagnostic X-Ray Contrast Agents in Combination witb Pharmaceutically Acceptable Clays;" U.S. Pat. No. 5,470,583 for "Method of Preparing Nannparticle Compositions Containing Charged Phospholipids to Reduce Aggregation;" U.S. Pat. No. 5,472,683 for "Nanoparticulate Diagnostic Mixed Carbamic Anhydrides as X-Ray Contrast Agents for Blood Pool and Lympbatic System Imaging," U.S. Pat. No. 5,500,204 for "Nanoparticulate Diagnostic Dimers as X-Ray Contrast Agents for Blood Pool and Lymphatic System Imaging;" U.S. Pat. No. 5,518,738 for "Nanoparticulate NSAID Formulations;" U.S. Pat. No. 5,521,218 for "Nanoparticulate Iododipamide Derivatives for Use as X-Ray Contrast Agents;" U.S. Pat. No. 5,525,328 for "Nanoparticulate Diagnostic Diatrizoxy Ester X-Ray Contrast Agents for Blood Pool and Lymphatic System Imaging;" U.S. Pat. No. 5,543,133 for "Process of Preparing X-Ray Contrast Compositions Containing Nanoparticles;" U.S. Pat. No. 5,552,160 for "Surface Modified NSAID Nanoparticles;" U.S. Pat. No. 5,560,931 for "Formulations of Compounds as Nanoparticulate Dispersions in Digestible Oils or Fatty Acids;" U.S. Pat. No. 5,565,188 for "Polyalkylene Block Copolymers as Surface Modifiers fnr Nanoparticles;" U.S. Pat. No. 5,569,448 for "Sulfated Non-ionic Block Copolymer Surfactant as Stabilizer Coatings for Nanoparticle Compositions;" U.S. Pat. No. 5,571,536 for "Formulations of Compounds as Nanoparticulate Dispersions in Digestible Oils or Fatty Acids;" U.S. Pat. No. 5,573,749 for "Nanoparticulate Diagnostic Mixed Carboxylic Anydrides as X-Ray Contrast Agents for Blood Pool and Lympbatic System Imaging;" U.S. Pat. Nn. 5,573,750 for "Diagnostic Imaging X-Ray Contrast Agents;" U.S. Pat. No 5,573,783 for "Redispersible Nanoparticulate Film Matrices With Protective Overcoats;" U.S. Pat. No. 5,580,579 for "Site-specific Adhesion Witbin the Gl Tract Using Nanoparticles Stabilized by High Molecular Weight, Linear Poly(ethylene Oxide) Polymers;" U.S Pat. No. 5,585,108 for "Formulations of Oral Gastrointestinal Therapeutic Agents in Combination with Pbarmaceutically Acceptable Clays;" U.S. Pat. No. 5,587,143 for "Butylene Oxide-Ethylene Oxide Block Copolymers Surfactants as Stabilizer Coatings for Nanoparticulate Compositions;" U.S. Pat. No. 5,591,456 for "Milled Naproxen witb Hydroxypropyl Cellulose as Dispersion Stabilizer," U.S. Pat. No. 5,593,657 for "Novel Barium Salt Formulations Stabilized by Non-innic and Anionic Stabilizers;" U.S. Pat. No. 5,622,938 for "Sugar Based Surfactant for Nanocrystals;" U.S. Pat. No. 5,628,981 for "Improved Formulations of Oral Gastrointestinal Diagnostic X-Ray Contrast Agents and Oral Gastrointestinal Therapeutic Agents;" U.S. Pat. No. 5,643,552 for "Nanoparticulate Diagnostic Mixed Carbonic Anhydrides as X-Ray Contrast Agents for Blood Pool and Lymphatic System Imaging;" U.S. Pat. No. 5,718,388 for "Continunus Method of Grinding Pharmaceutical Substances;" U.S. Pat. No. 5,718,919 for "Nanoparticles Containing the R(—)Enantiomer of Ibuprofen;" U.S. Pat. No. 5,747,001 for "Aerosols Containing Beclomethasone Nanoparticle Dispersions;" U.S. Pat. No. 5,834,025 for "Reduc-

tion of Intravenously Administered Nanoparticulate Formulation Induced Adverse Physiological Reactions;" U.S. Pat No. 6,045,829 "Nanocrystalline Formulations of Human Immunodeficiency Virus (HIV) Protease Inbibitors Using Cellulosic Surface Stabilizers;" U.S. Pat. No. 6,068,858 for "Methods of Making Nanocrystalline Formulations of Human Immunodeficiency Virus (HIV) Protease Inhibitors Using Cellulosic Surface Stabilizers;" U.S. Pat. No. 6,153,225 for "Injectable Formulations of Nanoparticulate Naproxen;" U.S. Pat. No. 6,165,506 for "New Solid Dose Form of Nanoparticulate Naproxen;" U.S. Pat. No. 6,221,400 for "Methods of Treating Mammals Using Nanocrystalline Formulations of Human Immunodeficiency Virus (IIIV) Protease Inhibitors;" U.S. Pat. No. 6,264,922 for "Nebulized Aerosols Containing Nanoparticle Dispersions;" U.S. Pat. No. 6,267,989 for "Methods for Preventing Crystal Growth and Particle Aggregation in Nanoparticle Compositions;" U.S. Pat. No. 6,270,806 for "Use of PEG-Derivatized Lipids as Surface Stabilizers for Nanoparticulate Compositions;" U.S. Pat. No. 6,316,029 for "Rapidly Disintegrating Solid Oral Dosage Form," U.S. Pat. No. 6,375,986 for "Solid Dose Nanoparticulate Compositions Comprising a Synergistic Combination of a Polymeric Surface Stabilizer and Dioctyl Sodium Sulfosuccinate," U.S. Pat. No. 6,428,814 for "Bioadhesive nanoparticulate compositions having cationic surface stabilizers;" U.S. Pat No.6,431,478 for "Small Scale Mill;" U.S. Pat. No. 6,432,381 for "Methods for Targeting Drug Delivery to the Upper and/or Lower Gastrointestinal Tract," and U.S. Pat. No. 6,592,903 for "Nanoparticulate Dispersions Comprising a Synergistic Combination of a Polymeric Surface Stabilizer and Dioctyl Sodium Sulfosuccinate," all of which are specifically incorporated by reference. In addition, U.S. patent application No. 20020012675 A1, published on Jan. 31, 2002, for "Controlled Release Nanoparticulate Compositions," and WO 02/098565 for "System and Method for Milling Materials," describe nanoparticulate active agent compositions, and are specifically incorporated by reference. None of these references describe nanoparticulate griseofulvin compositions.

[0008] Amorphous small particle compositions are described, for example, in U.S. Pat. No. 4,783,484 for "Particulate Composition and Use Thereof as Antimicrobial Agent;" U.S. Pat. No. 4,826,689 for "Method for Making Uniformly Sized Particles from Water-Insoluble Organic Compounds;" U.S. Pat. No. 4,997,454 for "Method for Making Uniformly-Sized Particles From Insoluble Compounds;" U.S. Pat. No. 5,741,522 for "Ultrasmall, Non-aggregated Porous Particles of Uniform Size for Entrapping Gas Bubbles Within and Methods;" and U.S. Pat. No. 5,776,496, for "Ultrasmall Porous Particles for Enhancing Ultrasound Back Scatter." These references do not describe nanoparticulate griseofulvin.

[0009] II. Background Regarding Griseofulvin

[0010] Griseofulvin is an antifungal antibiotic first isolated from a *Penicillium* species in 1939. The compound is insoluble in water, and slightly soluble in ethanol, methanol, acetone, benzene, $CHCl_3$, ethyl acetate, and acetic acid. Griseofulvin has the chemical formula $C_{17}H_{17}ClO_6$ and the following chemical structure:



[0011] *See The Merck Index*, 10th Edition, pp. 4433-34 (1983).

[0012] Griseofulvin is given orally to treat dermatophyte and ringworm infections of the scalp, hair, nails (fingernails and toenails), and skin caused by specific fungi. Griseofulvin is used to treat skin infections such as jock itch, athlete's foot, and ringworm. In particular, griseofulvin is useful in treating *Tinea capitis* (ringworm of the scalp), *Tinea corporis* (ringworm of the body), *Tinea pedis* (athlete's foot), *Tinea unguium* (ringworm of the nails), *Tinea cruris* (ringworm of the thigh), and *Tinea barbae* (barber's itch). Griseofulvin is also used to the following fungal infections of the hair, skin, and nails: *Trichophyton rubrum, Trichophyton tonsurans, Trichophyton mentagrophytes, Trichophyton interdigitalis, Trichophyton verrucosum, Trichophyton sulphureum, Trichophyton schoenleini, Trichophyton audouini, Trichophyton canis, Trichophyton gypseum, Trichophyton floccosum, Trichophyton megnini, Trichophyton gallinae*, and *Trichophyton crateriform*. This medication is sometimes prescribed for other uses, and it is used as an antifungal agent in veterinary medicine. Griseofulvin may be taken alone or used along with medicines that are applied to the skin for fungus infections.

[0013] Griseofulvin stops fungal cells dividing (i.e., it is fungistatic) but does not kill them outright. This means treatment needs to be continued for several weeks or months. Griseofulvin is generally well tolerated in children.

[0014] Griseofulvin is available as a tablet, capsule, and liquid to take by mouth. It is usually taken once a day or can be taken two to four times a day. Griseofulvin is usually taken for 2 to 4 weeks for skin infections, 4 to 6 weeks for hair and scalp infections, 4 to 8 weeks for foot infections, 3 to 4 months for fingernail infections, and at least 6 months for toenail infections.

[0015] The dose of griseofulvin will be different for different patients. In general, for adults the dose is 500 mg to 1 g daily, and for children it is 10-25 mg per kg body weight per day.

[0016] Griseofulvin absorption from the gastrointestinal tract varies considerably among individuals, mainly because of insolubility of the drug in aqueous media of the upper GI tract. *Physician's Desk Reference*, 57th Edition, p. 2445 (2003). The peak serum level found in fasting adults given 0.5 g occurs at about 4 hours and ranges between 0.5 and 2.0 mcg/mL.

[0017] Thus, griseofulvin is not very well absorbed from the gut. The drug should be taken after a meal or drink of milk as fat increases the absorption. The medication is carried into the skin by sweat and within a couple of weeks is concentrated in the outer skin layers.

[0018] Half tbe medication is cleared from the blood stream in 10 to 20 hours; the rest is eliminated in urine and faeces. This means the medication can be taken once daily. Griseofulvin should be continued until the fungal infectioo has completely gone because the medication is quickly cleared from skin and hair when it is stopped. Side effects of griseofulvin include headache; gastrointestinal upset, including nausea, vomiting, heartburn, cramps, flatulence, taste disturbaoce, diarrhea or loose stools, and furred tongue; thirst; fever; sore throat; skin rash (increased sun sensitivity); mouth soreness or irritation; urinary disturbance, including increased frequency and bed wetting; nervous system disturbance, including blurred vision, dizziness, depression, nightmares, faiotness, and fatigue; menstrual disturbance; and liver disturbance.

[0019] Griseofulvin has exhibited interactions with dietary supplements. In particular, adding 50 IU of vitamin E per day was reported to increase blood levels of griseofulvin within four weeks in children, allowing the drug dose to he cut in half. Reducing the amount of griseofulvin should decrease the likelihood of side effects.

[0020] Griseofulvin is currently produced by different companies. The microcystalline form is marketed as Grifulvin V™ (Ortho Dermatological). (Fulvicin U/F™ (Schering) and Grisactin™ (Wyeth-Ayerst), the other two microcrystalline products, are no longer marketed.) The ultramicrocrystalline form of griseofulvin is marketed as Gris-PEG™ (Pedinol). (Fulvicin P/G™ (Schering) and Grisactin Ultra™ (Wyeth-Ayerst), the other two ultramicrocrystalline products, are no longer marketed.)

[0021] U.S. Pat. No. 6,604,698 for "Media Milling," issued on Aug. 12, 2003, describes a process for preparing a dispersion of solid particles of a milled substrate, which can be griseofulvin, in a fluid carrier. The method comprises: (a) providing a plurality of large size milling media to the milling chamber of a media mill and forming a depth filter on an exit screen or separator in the milling chamber; (b) adding to the milling chamber a plurality of small size milling media, a conglomerate of a solid substance comprising a substrate to be milled, such as griseofulvin, and optionally one or more than one surface active substance, and a fluid carrier; (c) milling the conglomerate in the milling chamber to produce very small milled substrate product particles; and (d) continuously removing or separating the milled substrate particles suspended io the fluid carrier from the media through the depth filter. The media are retained in the mitling chamber.

[0022] U S. Pat. No. 6,569,463, for "Solid carriers for improved delivery of hydrophobic active ingredients in pharmaceutical compositions" issued on May 27, 2003, describes pharmaceutical compositions in the form of a solid carrier comprising a substrate and an eocapsulatioo coat on the substrate. The encapsulation coat comprises an admixture of a therapeutically effective amount of a hydrophobic pharmaceutical active ingredieot, such as griseofulvin, an effective solubilizing amount of at least one hydrophilic surfactaot, and a lipophilic additive selected from the group coosisting of lipophilic surfactants, triglycerides, and combinations thereof. The effective soluhilizing amount of the at least one hydrophilic surfactant is ao amount effective to partially or fully solubilize the pharmaceutical active ingredient in the encapsulatioo coat. This process is undesirahle as it requires solubilizing the active agent, which can change the properties of the active agent. Io addition, the solvents required to solubilize the active agent cao have undesirable side effects.

[0023] U.S. Pat. No. 5,785,976 for "Solid lipid particles, particles of bioactive agents and methods for the manufacture and use thereof," issued on Jul. 28, 1998, describes a process for makiog solid lipid particles (SLPs). The process comprises: (a) melting a solid agent, such as griseofulvin; (b) heating a dispersion medium to approximately the same temperature as the molten solid agent; (c) adding one or more highly mohile water-soluhle or dispersible stabilizers to the dispersion medium in such a way that the amount of highly mobile stabilizers is, after emulsification, sufficient to stahilize newly created surfaces during recrystallization; (d) homogenizing the melted agent and dispersion medium by high-pressure homogenization, micro-fluidization and/or ultrasonication; and (d) cooling the homogenized dispersion until solid particles are formed by recrystallizatinn of tbe dispersed agents. This process is undesirable as it requires melting the active agent, which can change the pmperties of the active agent.

[0024] U.S. Pat. Nos. 5,449,521 and 5,354,560, hoth for "Supported drugs with increased dissolution rate, and a process for their preparation," issued on Sep. 12, 1995, and Oct. 11, 1994, respectively, describe supported drugs having an increased dissolution rate and prepared hy a process comprising mixing the drug with the support material under dry conditions, co-grinding the mixture in a mill with its grinding chamber saturated with the vapour of one or more solvents able to solubilize the drug or to he adsorbed on the surface of the support material, vacuum-drying the product obtaioed, and sieving. The drugs obtained in this manner have a reduced heat of fusion, a reduced melting pnint, an iocreased dissolution rate and an increased solubilization kinetics. This process is undesirable as it requires solubilizing the active agent, which can change the properties of tbe active agent. In addition, the solvents required to solubilize tbe active agent can have undesirable side effects.

[0025] Finally, U.S. Pat. No. 5,705,194 for "Pharmaceutical compositions containing polyalkylene block copolymers which gel at physiological temperature," issued on Jan. 6, 1998, describes a pharmaceutical composition which gels at physiological temperature. The composition comprises a trihlock copolymer containing one or more polyoxyethylene blocks and one or more polyoxy (higher alkylene) blocks, wherein at least some of the blocks are linked together hy a linking group characterized in that the linking group is an oxymethyleoe group, and a therapeutic agent. The therapeutic agent, which can be griseofulvin, is present as (a) particles having an average size of less than about 400 nm and having the hlock copolymer adsorbed on the surface thereof, (b) a suspensioo in a solution of the block copolymer, or (c) as an aqueous solutioo in a solutioo of the block copolymer.

[0026] At conceotrations as low as 2.5% w/v in phosphate halance salt solutioo (PBS) or in water, the described block copolymers have gel points closc to physiological temperature (37.4° C.). The viscosity of these block copolymers at 3.5% and 5.5% in PBS changes ahruptly from less than 20 cps at room temperature to more than 1500 cps at physiological temperature, whitc the pH and osmolality of the

block copolymer solutions remain comparable to PBS. Thus, compositions containing these block copolymers can be administered (e.g., subcutaneously or orally) as low viscosity compositions at room temperature and, when they reach physiological temperature, will tend to gel.

[0027] Disadvantages of composition including such polyalkylene block copolymers include potential problems with IV administration, as well as potential difficulties in formulating the nanoparticulate composition into dosage forms for administration.

[0028] There is a need in the art for griseofulvin compositions which can improve clinical efficacy, reduce fed/fasted variability, and potentially reduce side effects. The present invention satisfies these needs.

SUMMARY OF THE INVENTION

[0029] The present invention relates to nanoparticulate griseofulvin compositions. The compositions comprise griseofulvin and at least one surface stabilizer. The nanoparticulate griseofulvin particles have an effective average particle size of less than about 2 microns.

[0030] Another aspect of the invention is directed to pharmaceutical compositions comprising a nanoparticulate griseofulvin composition of the invention. The pharmaceutical compositions preferably comprise griseofulvin, at least one surface stabilizer, and at least one pharmaceutically acceptable carrier, as well as any desired excipients. Advantages and properties of the compositions of the invention are described herein.

[0031] The invention further discloses a method of making a nanoparticulate griseofulvin composition. Such a method comprises contacting griseofulvin and at least one surface stabilizer for a time and under conditions sufficient to provide a nanoparticulate griseofulvin composition. The one or more surface stabilizers can be contacted with griseofulvin either before, preferably during, or after size reduction of the griseofulvin.

[0032] The present invention is also directed to methods of treating fungal infections using the nanoparticulate griseofulvin compositions. Such infections include, for example, such as dermatophyte and ringworm infections of the scalp, hair, nails (fingernails and toenails), and skin caused by specific fungi.

[0033] Both the foregoing general description and the following detailed description are exemplary and explanatory and are intended to provide further explanation of the invention as claimed. Other objects, advantages, and novel features will be readily apparent to those skilled in the art from the following detailed description of the invention.

DETAILED DESCRIPTION OF THE INVENTION

[0034] The present invention is directed to nanoparticulate griseofulvin compositions. The compositions comprise griseofulvin and at least one surface stabilizer that is preferably adsorbed on or associated with the surface of the drug. The nanoparticulate griseofulvin particles have an effective average particle size of less than about 2 microns.

[0035] As taught in the '684 patent, not every combination of surface stabilizer and active agent will result in a stable nanoparticulate composition. It was surprisingly discovered that stable nanoparticulate griseofulvin formulations can be made.

[0036] The current formulations of griseofulvin suffer from the following problems. (1) the poor solubility of the drug results in a relatively low bioavailability; (2) there is a significant variability in the fed/fasted absorption of the drug; (3) a wide variety of side effects are associated with the current dosage forms of the drug.

[0037] The present invention overcomes problems encountered with the prior art griseofulvin formulations. Specifically, the nanoparticulate griseofulvin formulations of the invention may offer the following advantages as compared to prior griseofulvin compositions: (1) faster onset of action; (2) a potential decrease in the frequency of dosing; (3) smaller doses of griseofulvin required to obtain the same pharmacological effect; (4) increased bioavailability; (5) an increased rate of dissolution; (6) improved performance characteristics for oral, intravenous, subcutaneous, or intramuscular injection, such as higher dose loading and smaller tablet or liquid dose volumes; (7) improved pharmacokinetic profiles, such as improved T_{max}, C_{max}, and AUC profiles; (8) substantially similar or bioequivalent pharmacokinetic profiles of the nanoparticulate griseofulvin compositions when administered in the fed versus the fasted state; (9) bioadhesive griseofulvin formulations, which can coat the gut or the desired site of application and be retained for a period of time, thereby increasing the efficacy of the drug as well as eliminating or decreasing the frequency of dosing; (10) high redispersibility of the nanoparticulate griseofulvin particles present in the compositions of the invention following administration; (11) the nanoparticulate griseofulvin compositions can be formulated in a dried form which readily redisperses; (12) low viscosity liquid nanoparticulate griseofulvin dosage forms can be made; (13) for liquid nanoparticulate griseofulvin compositions having a low viscosity—better subject compliance due to the perception of a lighter formulation which is easier to consume and digest; (14) for liquid nanoparticulate griseofulvin compositions having a low viscosity—ease of dispensing because one can use a cup or a syringe; (15) the nanoparticulate griseofulvin compositions can be used in conjunction with other active agents; (16) the nanoparticulate griseofulvin compositions can be sterile filtered; (17) the nanoparticulate griseofulvin compositions are suitable for parenteral administration; and (18) the nanoparticulate griseofulvin compositions do not require organic solvents or pH extremes.

[0038] A preferred dosage form of the invention is a solid dosage form, although any pharmaceutically acceptable dosage form can be utilized. Exemplary solid dosage forms include, but are not limited to, tablets, capsules, sachets, lozenges, powders, pills, or granules.

[0039] The dosage form of the invention can be, for example, a fast melt dosage form, controlled release dosage form, lyophilized dosage form, delayed release dosage form, extended release dosage form, pulsatile release dosage form, mixed immediate release and controlled release dosage form, or a combination thereof. A solid dose tablet formulation is preferred.

[0040] The present invention is described herein using several definitions, as set forth below and throughout the application.

[0041] As used herein, "about" will be understood by persons of ordinary skill in the art and will vary to some extent on the context in which it is used. If there are uses of the term which are not clear to persons of ordinary skill in the art given the context in which it is used, "about" will mean up to plus or minus 10% of the particular term.

[0042] "Conventional" or "non-nanoparticulate active agent" shall mean an active agent which is solubilized or which has an effective average particle size of greater than about 2 microns. Nanoparticulate active agents as defined herein have an effective average particle size of less than about 2 microns.

[0043] "Pharmaceutically acceptable" as used herein refers to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratin.

[0044] "Pharmaceutically acceptable salts" as used herein refers to derivatives wherein the parent compound is modified by making acid or base salts thereof. Examples of pharmaceutically acceptable salts include, but are not limited to, mineral or organic acid salts of basic residues such as amines; alkali or organic salts of acidic residues such as carboxylic acids; and the like. The pharmaceutically acceptable salts include the conventional non-toxic salts or the quaternary ammonium salts of the parent compound formed, for example, from non-toxic inorganic or organic acids. For example, such conventional non-toxic salts include those derived from inorganic acids such as hydrochloric, hydrobromic, sulfuric, sulfamic, phosphoric, nitric, and the like; and the salts prepared from organic acids such as acetic, propionic, succinic, glycolic, stearic, lactic, malic, tartaric, citric, ascorbic, pamoic, maleic, hydroxymaleic, phenylacetic, glutamic, benzoic, salicylic, sulfamilic, 2-acetoxybenzoic, fumaric, toluenesulfonic, methanesulfonic, ethane disulfonic, oxalic, isethionic, and the like.

[0045] "Poorly water soluble drugs" as used herein means those having a solubility of less than about 30 mg/ml, preferably less than about 20 mg/ml, preferably less than about 10 mg/ml, or preferably less than about 1 mg/ml. Such drugs tend to be eliminated from the gastrointestinal tract before being absorbed into the circulation.

[0046] As used herein with reference to stable drug particles, "stable" includes, but is not limited to, one or more of the following parameters: (1) that the griseofulvin particles do not appreciably flocculate or agglomerate due to interparticle attractive forces, or otherwise significantly increase in particle size over time; (2) that the physical structure of the griseofulvin particles is not altered over time, such as by conversion from an amorphous phase to crystalline phase; (3) that the griseofulvin particles are chemically stable; and/or (4) where the griseofulvin has not been subject to a heating step at or above the melting point of the griseofulvin in the preparation of the nanoparticles of the invention.

[0047] "Therapeutically effective amount" as used herein with respect to a drug dosage, shall mean that dosage that provides the specific pharmacological response for which the drug is administered in a significant number of subjects in need of such treatment. It is emphasized that "therapeutically effective amount," administered to a particular subject in a particular instance will not always be effective in treating the diseases described herein, even though such dosage is deemed a 'therapeutically effective amount' by those skilled in the art. It is to be further understood that drug dosages are, in particular instances, measured as oral dosages, or with reference to drug levels as measured in blood.

[0048] I. Preferred Characteristics of the Nanoparticulate Griseofulvin Compositions of the Invention

[0049] A. Increased Bioavailability, Frequency of Dosing, and Dosage Quantity

[0050] The nanoparticulate griseofulvin compositions of the invention may preferably exhibit increased bioavailability and require smaller doses as compared to prior non-nanoparticulate griseofulvin compositions administered at the same dose.

[0051] Any drug, including griseofulvin, can have adverse side effects. Thus, lower doses of griseofulvin that can achieve the same or better therapeutic effects as those observed with larger doses of non-nanoparticulate griseofulvin compositions are desired. Such lower doses may be realized with the nanoparticulate griseofulvin compositions of the invention because the nanoparticulate griseofulvin compositions may exhibit greater bioavailability as compared to non-nanoparticulate griseofulvin formulations, which means that smaller doses of griseofulvin are likely required to obtain the desired therapeutic effect.

[0052] The griseofulvin compositions of the invention may be administered less frequently and at lower doses in dosage forms such as liquid dispersions, powders, sprays, solid re-dispersable dosage forms, ointments, creams, etc. Exemplary types of formulations useful in the present invention include, but are not limited to, liquid dispersions, gels, aerosols (pulmonary and nasal), ointments, creams, solid dose forms, etc. of nanoparticulate griseofulvin. Lower dosages can be used because the small particle size of the griseofulvin particles ensure greater absorption, and in the case of bioadhesive nanoparticulate griseofulvin compositions, the griseofulvin is retained at the desired site of application for a longer period of time as compared to conventional griseofulvin dosage forms.

[0053] In one embodiment of the invention, the therapeutically effective amount of the nanoparticulate griseofulvin compositions is ⅙, ⅕, ¼, ⅓rd, or ½ of the therapeutically effective amount of a non-nanoparticulate griseofulvin composition.

[0054] Such lower doses are preferred as they may decrease or eliminate adverse effects of the drug. In addition, such lower doses decrease the cost of the dosage form and may increase patient compliance.

[0055] B. Pharmacokinetic Profiles of the Nanoparticulate Griseofulvin Compositions of the Invention

[0056] The invention also preferably provides griseofulvin compositions having a desirable pharmacokinetic profile when administered to mammalian subjects. The desirable pharmacokinetic profile of the griseofulvin compositions preferably includes, but is not limited to: (1) a T_{max} for griseofulvin, when assayed in the plasma of a mammalian subject following administration, that is preferably less than the T_{max} for a non-nanoparticulate griseofulvin formulation

administered at the same dosage; (2) a C_{max} for griseofulvin, when assayed in the plasma of a mammalian subject following administration, that is preferably greater than the C_{max} for a non-nanoparticulate griseofulvin formulation administered at the same dosage; and/or (3) an AUC for griseofulvin, when assayed in the plasma of a mammalian subject following administration, that is preferably greater than the AUC for a non-nanoparticulate griseofulvin formulation administered at the same dosage.

[0057] The desirable pharmacokinetic profile, as used herein, is the pharmacokinetic profile measured after the initial dose of griseofulvin. The compositions can be formulated in any way as described below and as known to those of skill in the art.

[0058] A preferred griseofulvin composition of the invention exhibits in comparative pharmacokinetic testing with a non-nanoparticulate griseofulvin formulation administered at the same dosage, a T_{max} not greater than about 90%, not greater than about 80%, not greater than about 70%, not greater than about 60%, not greater than about 50%, not greater than about 30%, not greater than about 25%, not greater than about 20%, not greater than about 15%, not greater than about 10%, or not greater than about 5% of the T_{max} exhibited by the non-nanoparticulate griseofulvin formulation.

[0059] This shorter T_{max} translates into a faster onset of therapeutic activity. The use of conventional formulations of griseofulvin is not ideal due to delayed onset of action. Specifically, conventional griseofulvin formulations exhibit a peak plasma concentration at 4 hours following administration. In contrast, the nanoparticulate griseofulvin compositions of the invention exhibit faster therapeutic effects.

[0060] A preferred griseofulvin composition of the invention exhibits in comparative pharmacokinetic testing with a non-nanoparticulate griseofulvin formulation of administered at the same dosage, a C_{max} which is at least about 50%, at least about 100%, at least about 200%, at least about 300%, at least about 400%, at least about 500%, at least about 600%, at least about 700%, at least about 800%, at least about 900%, at least about 1000%, at least about 1100%, at least about 1200%, at least about 1300%, at least about 1400%, at least about 1500%, at least about 1600%, at least about 1700%, at least about 1800%, or at least about 1900% greater than the C_{max} exhibited by the non-nanoparticulate griseofulvin formulation.

[0061] A preferred griseofulvin composition of the invention exhibits in comparative pharmacokinetic testing with a non-nanoparticulate griseofulvin formulation administered at the same dosage, an AUC which is at least about 25%, at least about 50%, at least about 75%, at least about 100%, at least about 125%, at least about 150%, at least about 175%, at least about 200%, at least about 225%, at least about 250%, at least about 275%, at least about 300%, at least about 350%, at least about 400%, at least about 450%, at least about 500%, at least about 550%, at least about 600%, at least about 750%, at least about 700%, at least about 750%, at least about 800%, at least about 850%, at least about 900%, at least about 950%, at least about 1000%, at least about 1050%, at least about 1100%, at least about 1150%, or at least about 1200% greater than the AUC exhibited by the non-nanoparticulate griseofulvin formulation.

[0062] Any formulation giving the desired pharmacokinetic profile is suitable for administration according to the present methods. Exemplary types of formulations giving such profiles are liquid dispersions, gels, aerosols, ointments, creams, solid dose forms, etc. of nanoparticulate griseofulvin.

[0063] C. The Pharmacokinetic Profiles of the Nanoparticulate Griseofulvin Compositions of the Invention are Preferably not Substantially Affected by the Fed or Fasted State of the Subject Ingesting the Compositions

[0064] The invention encompasses nanoparticulate griseofulvin compositions wherein preferably the pharmacokinetic profile of the griseofulvin is not substantially affected by the fed or fasted state of a subject ingesting the composition. This means that there is no substantial difference in the quantity of griseofulvin absorbed or the rate of griseofulvin absorption when the nanoparticulate griseofulvin compositions are administered in the fed versus the fasted state. Thus, the nanoparticulate griseofulvin compositions of the invention can substantially eliminate the effect of food on the pharmacokinetics of griseofulvin.

[0065] In another embodiment of the invention, the pharmacokinetic profile of the griseofulvin compositions of the invention, when administered to a mammal in a fasted state, is bioequivalent to the pharmacokinetic profile of the same griseofulvin composition administered at the same dosage, when administered to a mammal in a fed state. "Bioequivalency" is preferably established by a 90% Confidence Interval (CI) of between 0.80 and 1.25 for both C_{max} and AUC under U.S. Food and Drug Administration (USFDA) regulatory guidelines, or a 90% CI for AUC of between 0.80 to 1.25 and a 90% CI for C_{max} of between 0.70 to 1.43 under the European Medicines Evaluation Agency (EMEA) regulatory guidelines (T_{max} is not relevant for bioequivalency determinations under USFDA and EMEA regulatory guidelines).

[0066] Preferably the difference in AUC (e.g., absorption) of the nanoparticulate griseofulvin composition of the invention, when administered in the fed versus the fasted state, is less than about 100%, less than about 90%, less than about 80%, less than about 70%, less than about 60%, less than about 50%, less than about 40%, less than about 35%, less than about 30%, less than about 25%, less than about 20%, less than about 15%, less than about 10%, less than about 5%, or less than about 3%.

[0067] In addition, preferably the difference in C_{max} of the nanoparticulate griseofulvin composition of the invention, when administered in the fed versus the fasted state, is less than about 100%, less than about 90%, less than about 80%, less than about 70%, less than about 60%, less than about 50%, less than about 40%, less than about 35%, less than about 30%, less than about 25%, less than about 20%, less than about 15%, less than about 10%, less than about 5%, or less than about 3%.

[0068] Finally, preferably the difference in the T_{max} of the nanoparticulate griseofulvin compositions of the invention, when administered in the fed versus the fasted state, is less than about 100%, less than about 90%, less than about 80%, less than about 70%, less than about 60%, less than about 50%, less than about 40%, less than about 30%, less than about 20%, less than about 15%, less than about 10%, less than about 5%, less than about 3%, or essentially no difference.

[0069] Benefits of a dosage form that substantially eliminates the effect of food include an increase in subject convenience, thereby increasing subject compliance, as the subject does not need to ensure that they are taking a dose either with or without food.

[0070] D. Redispersibility Profiles of the Nanoparticulate Griseofulvin Compositions of the Invention

[0071] An additional feature of the nanoparticulate griseofulvin compositions of the invention is that the compositions redisperse such that the effective average particle size of the redispersed griseofulvin particles is less than about 2 microns. This is significant, as if upon administration the nanoparticulate griseofulvin particles present in the compositions of the invention did not redisperse to a substantially nanoparticulate particle size, then the dosage form may lose the benefits afforded by formulating griseofulvin into a nanoparticulate particle size.

[0072] This is because nanoparticulate griseofulvin compositions benefit from the small particle size of griseofulvin; if the nanoparticulate griseofulvin particles do not redisperse into the small particle sizes upon administration, then "clumps" or agglomerated griseofulvin particles are formed. With the formation of such agglomerated particles, the bioavailability of the dosage form may fall.

[0073] Moreover, the nanoparticulate griseofulvin compositions of the invention exhibit dramatic redispersion of the griseofulvin particles upon administration to a mammal, such as a human or animal, as demonstrated by reconstitution in a biorelevant aqueous media. Such biorelevant aqueous media can be any aqueous media that exhibit the desired ionic strength and pH, which form the basis for the biorelevance of the media. The desired pH and ionic strength are those that are representative of physiological conditions found in the human body. Such biorelevant aqueous media can be, for example, aqueous electrolyte solutions or aqueous solutions of any salt, acid, or base, or a combination thereof, which exhibit the desired pH and ionic strength.

[0074] Biorelevant pH is well known in the art. For example, in the stomach, the pH ranges from slightly less than 2 (but typically greater than 1) up to 4 or 5. In the small intestine the pH can range from 4 to 6, and in the colon it can range from 6 to 8. Biorelevant ionic strength is also well known in the art. Fasted state gastric fluid has an ionic strength of about 0.1M while fasted state intestinal fluid has an ionic strength of about 0.14. See e.g., Lindahl et al., "Characterization of Fluids from the Stomach and Proximal Jejunum in Men and Women," *Pharm. Res.*, 14 (4): 497-502 (1997).

[0075] It is believed that the pH and ionic strength of the test solution is more critical than the specific chemical content. Accordingly, appropriate pH and ionic strength values can be obtained through numerous combinations of strong acids, strong bases, salts, single or multiple conjugate acid-base pairs (i.e., weak acids and corresponding salts of that acid), monoprotic and polyprotic electrolytes, etc.

[0076] Representative electrolyte solutions can be, but are not limited to, HCl solutions, ranging in concentration from about 0.001 to about 0.1 M, and NaCl solutions, ranging in concentration from about 0.001 to about 0.1 M, and mixtures thereof. For example, electrolyte solutions can be, but are not limited to, about 0.1 M HCl or less, about 0.01 M HCl or less, about 0.001 M HCl or less, about 0.1 M NaCl or less, about 0.01 M NaCl or less, about 0.001 M NaCl or less, and mixtures thereof. Of these electrolyte solutions, 0.01 M HCl and/or 0.1 M NaCl, are most representative of fasted human physiological conditions, owing to the pH and ionic strength conditions of the proximal gastrointestinal tract.

[0077] Electrolyte concentrations of 0.001 M HCl, 0.01 M HCl, and 0.1 M HCl correspond to pH 3, pH 2, and pH 1, respectively. Thus, a 0.01 M HCl solution simulates typical acidic conditions found in the stomach. A solution of 0.1 M NaCl provides a reasonable approximation of the ionic strength conditions found throughout the body, including the gastrointestinal fluids, although concentrations higher than 0.1 M may be employed to simulate fed conditions within the human GI tract.

[0078] Exemplary solutions of salts, acids, bases or combinations thereof, which exhibit the desired pH and ionic strength, include but are not limited to phosphoric acid/phosphate salts+sodium, potassium and calcium salts of chloride, acetic acid/acetate salts+sodium, potassium and calcium salts of chloride, carbonic acid/bicarbonate salts+sodium, potassium and calcium salts of chloride, and citric acid/citrate salts+sodium, potassium and calcium salts of chloride.

[0079] In other embodiments of the invention, the redispersed griseofulvin particles of the invention (redispersed in an aqueous, biorelevant, or any other suitable media) have an effective average particle size of less than about 1900 nm, less than about 1800 nm, less than about 1700 nm, less than about 1600 nm, less than about 1500 nm, less than about 1400 nm, less than about 1300 nm, less than about 1200 nm, less than about 1100 nm, less than about 1000 nm, less than about 900 nm, less than about 800 nm, less than about 700 nm, less than about 600 nm, less than about 500 nm, less than about 400 nm, less than about 300 nm, less than about 250 nm, less than about 200 nm, less than about 150 nm, less than about 100 nm, less than about 75 nm, or less than about 50 nm, as measured by light-scattering methods, microscopy, or other appropriate methods.

[0080] Redispersibility can be tested using any suitable means known in the art. See e.g., the example sections of U.S. Pat. No. 6,375,986 for "Solid Dose Nanoparticulate Compositions Comprising a Synergistic Combination of a Polymeric Surface Stabilizer and Dioctyl Sodium Sulfosuccinate."

[0081] E. Bioadhesive Nanoparticulate Griseofulvin Compositions

[0082] Bioadhesive nanoparticulate griseofulvin compositions of the invention comprise at least one cationic surface stabilizer, which are described in more detail below. Bioadhesive formulations of griseofulvin exhibit exceptional bioadhesion to biological surfaces, such as mucous and skin.

[0083] In the case of bioadhesive nanoparticulate griseofulvin compositions, the term "bioadhesion" is used to describe the adhesion between the nanoparticulate griseofulvin compositions and a biological substrate (i.e., gastrointestinal mucin, lung tissue, nasal mucosa, etc.). See e.g., U.S. Pat. No. 6,428,814 for "Bioadhesive Nanoparticulate Compositions Having Cationic Surface Stabilizers," which is specifically incorporated by reference.

[0084] The bioadhesive griseofulvin compositions of the inventioo are useful in any situation in wbieb it is desirable to apply tbe compositions to a biological surface. The bioadhesive griseofulvin compositions preferably coat the targeted surface in a continuous and uniform film tbat is invisible to the naked human eye.

[0085] A bioadhesive nanoparticulate grisenfulviu composition slows the transit of the composition, and some griseofulvin particles would also most likely adbere to tissue other than the mucous cells and therefore give a prolonged exposure to griseofulvin, thereby increasing absorption and the bioavailability of the adminstered dosage.

[0086] F. Low Viscosity

[0087] A liquid dosage form of a conventional microcrystalline or non-nanoparticulate griseofulvin composition would be expected to be a relatively large volume, highly viscous substance which would not be well accepted by patient populations. Moreover, viscous solutions can be problematic in parenteral administration because these solutions require a slow syringe push and can stick to tubing. In addition, conventional formulations of poorly water-soluble active agents, such as griseofulvin, tend to be unsafe for intravenous administration techniques, which are used primarily in conjunction with higbly water-soluble substances.

[0088] Liquid dosage forms of the nanoparticulate griseofulvin compositions of the invention provide significant advantages over a liquid dosage form of a conventional microcrystalline or solubilized griseofulvin compnsition. The low viscosity and silky texture of liquid dosage forms of the nanoparticulate griseofulvin compositions of the inventioo result in advantages in both preparation and use. These advantages include, for example: (1) better subject compliance due to the perception of a lighter formulation which is easier to consume and digest; (2) ease of dispensing because nne can use a cup or a syringe; (3) potential for formulating a higher concentration of griseofulvin resulting in a smaller dosage vnlume and thus less vnlume for the subject to consume; and (4) easier overall formulation concerns.

[0089] Liquid griseofulvin dosage fnrms that are easier to consume are especially important when considering juvenile patients, terminally ill patients, and elderly patients. Viscous or gritty formulations, and those that require a relatively large dosage volume, arc not well tolerated by these patient populations. Liquid oral dosage forms can he particularly preferably for patient populations who bave difficulty consumiog tablets, such as iofants and the elderly.

[0090] The viscosities of liquid dosage forms of nanoparticulate griseofulvin accordiog to the invention are preferably less than about 1/200, less than about 1/175, less than about 1/150, less than about 1/125, less than about 1/100, less than about 1/75, less tban about 1/50, or less tban about 1/25 of a liquid oral dosage form of a non-nanoparticulate griseofulvin composition, at about the same concentration per ml of griseofulvin.

[0091] Typically the viscosity of liquid nanoparticulate griseofulvin dosage forms of tbe invention, at a sbear rate of 0.1 (1/s) measured at 20° C., is from about 2000 mPa s to about 1 mPa s, from about 1900 mPa.s to about 1 mPa.s, from about 1800 mPa.s to about 1 mPa.s , from about 1700 mPa.s to about 1 mPa.s , from abnut 1600 mPa.s to about 1 mPa.s , from about 1500 mPa.s to about 1 mPa.s , from about 1400 mPa.s to about 1 mPa.s , from about 1300 mPa.s to about 1 mPa.s , from about 1200 mPa.s to about 1 mPa.s , from about 1100 mPa.s to about 1 mPa.s , from about 1000 mPa.s to about 1 mPa.s , from about 900 mPa.s to about 1 mPa.s , from about 800 mPa.s tn abnut 1 mPa.s , from about 700 mPa.s to about 1 mPa.s , from about 600 mPa.s to about 1 mPa.s , from about 500 mPa.s to abnut 1 mPa.s , from about 400 mPa.s to about 1 mPa.s , from about 300 mPa.s to about 1 mPa.s , from about 200 mPa.s to about 1 mPa s , from about 175 mPa.s to abnut 1 mPa.s , from about 150 mPa.s to about 1 mPa.s , from about 125 mPa.s to about 1 mPa.s , from about 100 mPa.s to about 1 mPa.s , from about 75 mPa.s to about 1 mPa s , from about 50 mPa.s to about 1 mPa.s , from about 25 mPa.s to about 1 mPa.s , from about 15 mPa.s to about 1 mPa.s , from about 10 mPa.s to about 1 mPa.s , or from about 5 mPa.s to about 1 mPa.s . Such a viscosity is much more attractive for subject consumption and may lead to better overall subject compliance.

[0092] Viscosity is concentration and temperature dependent. Typically, a higher concentration results in a higher viscosity, while a higher temperature results in a lower viscosity. Viscosity as defined above refers to measurements taken at about 20° C. (The viscosity of water at 20° C. is 1 mPa.s.) The invention encompasses equivalent viscosities measured at different temperatures.

[0093] Another important aspect of tbe invention is that the nanoparticulate griseofulvin compositions of the invention, formulated into a liquid dosage form, are oot turbid. "Turbid," as used herein refers to the property of particulate matter that can be seen with the naked eye or that which can be felt as "gritty." The nanoparticulate griseofulvin compositions of the invention, formulated into a liquid dosage form, can be poured out of or extracted from a container as easily as water, whereas a liquid dosage form of a non-nanoparticulate or solubilized griseofulvin is expected to exhibit notably more "sluggish" characteristics.

[0094] The liquid formulations of this invention can be formulated for dosages in any volume but preferably equivalent or smaller volumes than a liquid dosage form of a non-nanoparticulate griseofulvin composition.

[0095] G. Sterile Filtered Nanoparticulate Griseofulvin Compositions

[0096] The nanoparticulate griseofulvin compositions of tbe invention can be sterile filtered. This obviates the need for beat sterilization, which can harm or degrade griseofulvin, as well as result in crystal growth and particle aggregation.

[0097] Sterile filtration can be difficult because of the required small particle size of the composition. Filtration is an effective method for sterilizing homogeneous solutions wben the membrane filter pore size is less than or equal to about 0.2 microns (200 nm) because a 0.2 micron filter is sufficient to remove essentially all bacteria. Sterile filtration is normally not used to sterilize suspensions of micron-sized griseofulvin because the griseofulvin particles are too large to pass through the membrane pores.

[0098] A sterile nanoparticulate griseofulvin dosage form is particularly useful in treating immunocompromised patients, infants or juvenile patients, aod the elderly, as these

patient groups are the most susceptible to infection caused by a non-sterile liquid dosage form.

[0099] Because the nanoparticulate griseofulvin compositions of the invention, formulated into a liquid dosage form, can be sterile filtered, and because the compositions can have a very small griseofulvin effective average particle size, the compositions are suitable for parenteral administration.

[0100] II. Combination Pharmacokinetic Profile Compositions

[0101] In yet another embodiment of the invention, a first nanoparticulate griseofulvin composition providing a desired pharmacokinetic profile is co-administered, sequentially administered, or combined with at least one other griseofulvin composition that generates a desired different pharmacokinetic profile. More than two griseofulvin compositions can be co-administered, sequentially administered, or combined. While the first griseofulvin composition has a nanoparticulate particle size, the additional one or more griseofulvin compositions can be nanoparticulate, solubilized, or have a microparticulate particle size.

[0102] For example, a first griseofulvin composition can have a nanoparticulate particle size, conferring a short T_{max} and typically a higher C_{max}. This first griseofulvin composition can be combined, co-administered, or sequentially administered with a second composition comprising: (1) griseofulvin having a larger (but still nanoparticulate as defined herein) particle size, and therefore exhibiting slower absorption, a longer T_{max}, and typically a lower C_{max}; or (2) a microparticulate or solubilized griseofulvin composition, exhibiting a longer T_{max}, and typically a lower C_{max}.

[0103] The second, third, fourth, etc., griseofulvin compositions can differ from the first, and from each other, for example: (1) in the effective average particle sizes of griseofulvin; or (2) in the dosage of griseofulvin. Such a combination composition can reduce the dose frequency required.

[0104] If the second griseofulvin composition has a nanoparticulate particle size, then preferably the griseofulvin particles of the second composition have at least one surface stabilizer associated with the surface of the drug particles. The one or more surface stabilizers can be the same as or different from the surface stabilizer(s) present in the first griseofulvin composition.

[0105] Preferably where co-administration of a "fast-acting" formulation and a "longer-lasting" formulation is desired, the two formulations are combined within a single composition, for example a dual-release composition.

[0106] I. Combination Active Agent Compositions

[0107] The invention encompasses the nanoparticulate griseofulvin compositions of the invention formulated or co-administered with one or more non-griseofulvin active agents. Methods of using such combination compositions are also encompassed by the invention. The non-griseofulvin active agents can be present in a crystalline phase, an amorphous phase, a semi-crystalline phase, a semi-amorphous phase, or a mixture thereof.

[0108] The compound to be administered in combination with a nanoparticulate griseofulvin composition of the invention can be formulated separately from the nanoparticulate griseofulvin composition or co-formulated with the nanoparticulate griseofulvin composition. Where a nanoparticulate griseofulvin composition is co-formulated with a second active agent, the second active agent can be formulated in any suitable manner, such as immediate-release, rapid-onset, sustained-release, or dual-release form.

[0109] Such non-griseofulvin active agents can be, for example, a therapeutic agent. A therapeutic agent can be a pharmaceutical agent, including a biologic. The active agent can be selected from a variety of known classes of drugs, including, for example, amino acids, proteins, peptides, nucleotides, anti-obesity drugs, central nervous system stimulants, carotenoids, corticosteroids, elastase inhibitors, anti-fungals, oncology therapies, anti-emetics, analgesics, cardiovascular agents, anti-inflammatory agents, such as NSAIDs and COX-2 inhibitors, anthelmintics, anti-arrhythmic agents, antibiotics (including penicillins), anticoagulants, antidepressants, antidiabetic agents, antiepileptics, antihistamines, antihypertensive agents, antimuscarinic agents, antimycobacterial agents, antineoplastic agents, immunosuppressants, antithyroid agents, antiviral agents, anxiolytics, sedatives (hypnotics and neuroleptics), astringents, alpha-adrenergic receptor blocking agents, beta-adrenoceptor blocking agents, blood products and substitutes, cardiac inotropic agents, contrast media, corticosteroids, cough suppressants (expectorants and mucolytics), diagnostic agents, diagnostic imaging agents, diuretics, dopaminergics (antiparkinsonian agents), haemostatics, immunological agents, lipid regulating agents, muscle relaxants, parasympathomimetics, parathyroid calcitonin and biphosphonates, prostaglandins, radio-pharmaceuticals, sex hormones (including steroids), anti-allergic agents, stimulants and anoretics, sympathomimetics, thyroid agents, vasodilators, and xanthines.

[0110] Examples of representative active agents useful in this invention include, but are not limited to, acyclovir, alprazolam, altretamine, amiloride, amiodarone, benztropine mesylate, bupropion, cabergoline, candesartan, cerivastatin, chlorpromazine, ciprofloxacin, cisapride, clarithromycin, clonidine, clopidogrel, cyclobenzaprine, cyproheptadine, delavirdine, desmopressin, diltiazem, dipyridamole, dolasetron, enalapril maleate, enalaprilat, famotidine, felodipine, furazolidone, irbesartan, ketoconazole, lansoprazole, loratadine, loxapine, mebendazole, mercaptopurine, milrinone lactate, minocycline, mitoxantrone, nelfinavir mesylate, nimodipine, norfloxacin, olanzapine, omeprazole, penciclovir, pimozide, tacolimus, quazepam, raloxifene, rifabutin, rifampin, risperidone, rizatriptan, saquinavir, sertraline, sildenafil, acetyl-sulfisoxazole, temazepam, thiabendazole, thioguanine, trandolapril, triamterene, trimetrexate, troglitazone, trovafloxacin, verapamil, vinblastine sulfate, mycophenolate, atovaquone, atovaquone, proguanil, ceftazidime, cefuroxime, etoposide, terbinafine, thalidomide, fluconazole, amsacrine, dacarbazine, teniposide, and acetylsalicylate.

[0111] A description of these classes of active agents and a listing of species within each class can be found in Martindale's *The Extra Pharmacopoeia*, 31st Edition (The Pharmaceutical Press, London, 1996), specifically incorporated by reference. The active agents are commercially available and/or can be prepared by techniques known in the art. Exemplary nutraceuticals or dietary supplements

include, but are not limited to, lutein, folic acid, fatty acids (e.g., DHA and ARA), fruit and vegetable extracts, vitamin and mineral supplements, phosphatidylserine, lipoic acid, melatonin, glucosamine/chondroitin, Aloe Vera, Guggul, glutamine, amino acids (e.g., arginine, isoleucine, leucine, leucine, lysine, methionine, phenylanine, threonine, tryptophan, and valine), green tea, lycopene, whole foods, food additives, herbs, phytonutrients, antioxidants, flavonoid constituents of fruits, evening primrose oil, flax seeds, fish and marine animal oils, and probiotics. Nutraceuticals and dietary supplements also include bio-engineered foods genetically engineered to have a desired property, also known as "pharmafoods." Exemplary nutraceuticals and dietary supplements are disclosed, for example, in Roberts et al., *Nutraceuticals: The Complete Encyclopedia of Supplements, Herbs, Vitamins, and Healing Foods* (American Nutraceutical Association, 2001), which is specifically incorporated by reference. Dietary supplements and nutraceuticals are also disclosed in *Physicians' Desk Reference for Nutritional Supplements*, 1st Ed. (2001) and *The Physicians' Desk Reference for Herbal Medicines*, 1st Ed. (2001), both of which are also incorporated by reference. A nutraceutical or dietary supplement, also known as a phytochemical or functional food, is generally any one of a class of dietary supplements, vitamins, minerals, herbs, or healing foods that have medical or pharmaceutical effects on the body.

[0112] In a preferred embodiment of the invention, the griseofulvin compositions of the invention are co-administered or combined with at least one other antifungal agent.

[0113] In another preferred embodiment of the invention, the griseofulvin compositions of the invention are co-administered or combined with vitamin E.

[0114] J. Miscellaneous Benefits of the Nanoparticulate Griseofulvin Compositions of the Invention

[0115] The nanoparticulate griseofulvin compositions preferably exhibit an increased rate of dissolution as compared to microcrystalline or non-nanoparticulate forms of griseofulvin. In addition, the nanoparticulate griseofulvin compositions preferably exhibit improved performance characteristics for oral, intravenous, subcutaneous, or intramuscular injection, such as higher dose loading and smaller tablet or liquid dose volumes. Moreover, the nanoparticulate griseofulvin compositions of the invention do not require organic solvents or pH extremes.

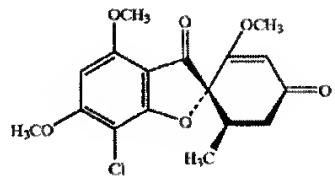
[0116] II. Griseofulvin Compositions

[0117] The invention provides compositions comprising nanoparticulate griseofulvin particles and at least one surface stabilizer. The surface stabilizers are preferably associated with the surface of the griseofulvin particles. Surface stabilizers useful herein do not chemically react with the griseofulvin particles or itself. Preferably, individual molecules of the surface stabilizer are essentially free of intermolecular cross-linkages. The compositions can comprise two or more surface stabilizers.

[0118] The present invention also includes nanoparticulate griseofulvin compositions together with one or more non-toxic physiologically acceptable carriers, adjuvants, or vehicles, collectively referred to as carriers. The compositions can be formulated for parenteral injection (e.g., intravenous, intramuscular, or subcutaneous), oral administration (in solid, liquid, or aerosol (i.e., pulmonary) form), vaginal, nasal, rectal, ocular, local (powders, creams, ointments or drops), buccal, intracisternal, intraperitoneal, topical administration, and the like.

[0119] A. Griseofulvin Particles

[0120] Griseofulvin as used herein includes the compound having the chemical formula $C_{17}H_{17}ClO_6$ and the following chemical structure:



[0121] and salts thereof. *See The Merck Index*, 10th Edition, pp.4433-34 (1983).

[0122] Griseofulvin can be in a crystalline phase, an amorphous phase, a semi-crystalline phase, a semi-amorphous phase, or a mixture thereof.

[0123] B. Surface Stabilizers

[0124] The choice of a surface stabilizer for griseofulvin is non-trivial and required extensive experimentation to realize a desirable formulation. Accordingly, the present invention is directed to the surprising discovery that nanoparticulate griseofulvin compositions can be made.

[0125] Combinations of more than one surface stabilizer can be used in the invention. Useful surface stabilizers that can be employed in the invention include, but are not limited to, known organic and inorganic pharmaceutical excipients. Such excipients include various polymers, low molecular weight oligomers, natural products, and surfactants. Surface stabilizers include nonionic, anionic, cationic, zwitterionic, and ionic surfactants.

[0126] Representative examples of other useful surface stabilizers include hydroxypropyl methylcellulose, hydroxypropylcellulose, polyvinylpyrrolidone, sodium lauryl sulfate, dioctylsulfosuccinate, gelatin, casein, lecithin (phosphatides), dextran, gum acacia, cholesterol, tragacanth, stearic acid, benzalkonium chloride, calcium stearate, glycerol monostearate, cetostearyl alcohol, cetomacrogol emulsifying wax, sorbitan esters, polyoxyethylene alkyl ethers (e.g., macrogol ethers such as cetomacrogol 1000), polyoxyethylene castor oil derivatives, polyoxyethylene sorbitan fatty acid esters (e.g., the commercially available Tweens® such as e.g., Tween® and Tween 80® (ICI Speciality Chemicals)); polyethylene glycols (e.g., Carbowaxs 3550® and 934® (Union Carbide)), polyoxyethylene stearates, colloidal silicon dioxide, phosphates, carboxymethylcellulose calcium, carboxymethylcellulose sodium, methylcellulose, hydroxyethylcellulose, hydroxypropylmethylcellulose phthalate, noncrystalline cellulose, magnesium aluminium silicate, triethanolamine, polyvinyl alcohol (PVA), 4-(1,1,3,3-tetramethylbutyl)-phenol polymer with ethylene oxide and formaldehyde (also known as tyloxapol, superione, and triton), poloxamers (e.g., Pluronics F68® and F108®, which

are block copolymers of ethylene oxide and propylene oxide); poloxamines (e.g., Tetronic 908®, also known as Poloxamine 908®, which is a tetrafunctional block copolymer derived from sequential addition of propylene oxide and ethylene oxide to ethylenediamine (BASF Wyandotte Corporation, Parsippany, N.J.)); Tetronic 1508® (T-1508) (BASF Wyandotte Corporation), Tritons X-200®, which is an alkyl aryl polyether sulfonate (Rohm and Haas); Crodestas F-110®, which is a mixture of sucrose stearate and sucrose distearate (Croda Inc.); p-isononylphenoxypoly-(glycidol), also known as Olin-10G® or Surfactant 10-G® (Olin Chemicals, Stamford, Conn.); Crodestas SL-40® (Croda, Inc.); and SA9OHCO, which is $C_{18}H_{37}CH_2(CON(CH_3)—CH_2(CHOH)_4(CH_2OH)_2$ (Eastman Kodak Co.); decanoyl-N-methylglucamide; n-decyl β-D-glucopyranoside, n-decyl β-D-maltopyranoside; n-dodecyl β-D-glucopyranoside; n-dodecyl β-D-maltoside; heptanoyl-N-methylglucamide; n-heptyl-β-D-glucopyranoside; n-heptyl β-D-thioglucoside; n-hexyl β-D-glucopyranoside; nonanoyl-N-methylglucamide; n-noyl β-D-glucopyranoside; octanoyl-N-methylglucamide; n-octyl-β-D-glucopyranoside; octyl β-D-thioglucopyranoside; PEG-derivatized phospholipid, PEG-derivatized cholesterol, PEG-derivatized cholesterol derivative, PEG-derivatized vitamin A, PEG-derivatized vitamin E, lysozyme, random copolymers of vinyl pyrrolidone and vinyl acetate, and the like.

[0127] Depending upon the desired method of administration, bioadhesive formulations of nanoparticulate griseofulvin can he prepared hy selecting one or more cationic surface stabilizers that impart hioadhesive properties to the resultant composition. Useful cationic surface stahilizers are described helow.

[0128] Examples of useful cationic surface stahilizers include, but are not limited to, polymers, hiopolymers, polysaccharides, cellulosics, alginates, phospholipids, and nonpolymeric compounds, such as zwitterionic stahilizers, poly-n-methylpyridinium, anthryul pyridinium chloride, cationic phospholipids, chitosan, polylysine, polyvinylimidazole, polybrene, polymethylmethacrylate trimethylammoniumbromide bromide (PMMTMABr), hexyldesyltrimethylammonium bromide (HDMAB), polyvinylpyrrolidone-2-dimethylaminoethyl methacrylate dimethyl sulfate, 1,2 Dipalmitoyl-sn-Glycero-3-Phosphoethanolamine-N-[Amino(Polyethylene Glycol)2000] (sodium salt) (also known as DPPE-PEG(2000)-Amine Na) (Avanti Polar Lipids, Alahaster, Al), Poly(2-methacryloxyethyl trimethylammonium bromide) (Polysciences, Inc., Warrington, Pa.) (also known as S1001), poloxamines such as Tetronic 908®, also known as Poloxamine 908®, which is a tetrafunctional hlock copolymer derived from sequential addition of propylene oxide and ethylene oxide to ethylenediamine (BASF Wyandotte Corporation, Parsippany, N.J.), lysozyme, long-chain polymers such as alginic acid, carrageenan (FMC Corp.), and POLYOX (Dow, Midland, Mich.).

[0129] Other useful cationic stahilizers include, but are not limited to, cationic lipids, sulfonium, phosphonium, and quarternary ammoonium compounds, such as stearyltrimethylammonium chloride, benzyl-di(2-chlorocthyl)ethylammonium bromide, coconut trimethyl ammonium chloride or bromide, coconut methyl dihydroxyethyl ammonium chloride or bromide, decyl triethyl ammonium chloride, decyl dimethyl hydroxyethyl ammonium chloride or hromide, C_{12-15}dimethyl hydroxyethyl ammonium chloride or bromide, coconut dimethyl hydroxyethyl ammonium chloride or bromide, myristyl trimethyl ammonium methyl sulphate, lauryl dimethyl benzyl ammonium chloride or bromide, lauryl dimethyl(ethenoxy)$_4$ ammonium chloride or bromide, N-alkyl (C_{12-18})dimethylbenzyl ammonium chloride, N-alkyl(C_{14-18})dimethyl-benzyl ammonium chloride, N-tetradecylidmethylbenzyl ammonium chloride monohydrate, dimethyl didecyl ammonium chloride, N-alkyl and (C_{12-14})dimethyl 1-naphthylmethyl ammonium chloride, trimethylammonium halide, alkyl-trimethylammonium salts and dialkyl-dimethylammonium salts, lauryl trimethyl ammonium chloride, ethoxylated alkyamidoalkyldialkylammonium salt and/or an ethoxylated trialkyl ammonium salt, dialkylbenzene dialkylammonium chloride, N-didecyldimethyl ammonium chloride, N-tetradecyldimethylbenzyl ammonium, chloride monohydrate, N-alkyl(C_{12-14})dimethyl 1-naphthylmethyl ammonium chloride and dodecyldimethylbenzyl ammonium chloride, dialkyl benzenealkyl ammonium chloride, lauryl trimethyl ammonium chloride, alkylhenzyl methyl ammonium chloride, alkyl henzyl dimethyl ammonium hromide, C_{12}, C_{15}, C_{17} trimethyl ammonium hromides, dodecylbenzyl triethyl ammonium chloride, polydiallyldimethylammonium chloride (DADMAC), dimethyl ammonium chlorides, alkyldimethylammonium halogenides, tricetyl methyl ammonium chloride, decyltrimethylammonium hromide, dodecyltriethylammonium bromide, tetradeceyltrimethylammonium hromide, methyl trioctylammonium chloride (ALIQUAT 336™), POLYQUAT 10™, tetrahutylammonium hromide, benzyl trimethylammonium bromide, choline esters (such as choline esters of fatty acids), henzalkonium chloride, stearalkonium chloride compounds (such as stearyltrimonium chloride and Di-stearyldimonium chloride), cetyl pyridinium hromide or chloride, halide salts of quaternized polyoxyethylalkylamines, MIRAPOL™ and ALKAQUA™ (Alkaril Chemical Company), alkyl pyridinium salts; amines, such as alkylamines, dialkylamines, alkanolamines, polyethylenepolyamines, N,N-dialkylaminoalkyl acrylates, and vinyl pyridine, amine salts, such as lauryl amine acetate, stearyl amine acetate, alkylpyridinium salt, and alkylimidazolium salt, and amine oxides; imide azolinium salts; protonated quaternary acrylamides; methylated quaternary polymers, such as poly [diallyl dimethylammonium chloride] and poly-[N-methyl vinyl pyridinium chloride]; and cationic guar.

[0130] Such exemplary cationic surface stabilizers and other useful cationic surface stahilizers are described io J. Cross and E. Singer, *Cationic Surfactants: Analytical and Biological Evaluation* (Marcel Dekker, 1994); P. and D. Rubingh (Editor), *Cationic Surfactants: Physical Chemistry* (Marcel Dekker, 1991); and J. Richmond, *Cationic Surfactants: Organic Chemistry*, (Marcel Dekker, 1990).

[0131] Nonpolymeric cationic surface stabilizers are any nonpolymeric compound, such as benzalkonium chloride, a carhonium compound, a phosphonium compound, an oxonium compound, a halonium compound, a cationic organometallic compound, a quarternary phosphorous compound, a pyridinium compound, an anilinium compound, an ammonium compound, a hydroxylammonium compound, a primary ammonium compound, a secondary ammonium compound, a tertiary ammonium compound, and quarternary ammonium compounds of the formula $NR_1R_2R_3R_4^{(+)}$. For compounds of the formula $NR_1R_2R_3R_4^{(+)}$:

[0132] (i) none of R_1—R_4 are CH_3;

[0133] (ii) one of R_1—R_4 is CH_3;

[0134] (iii) three of R_1—R_4 are CH_3;

[0135] (iv) all of R_1—R_4 are CH_3;

[0136] (v) two of R_1—R_4 are CH_3, one of R_1—R_4 is $C_6H_5CH_2$, and one of R_1—R_4 is an alkyl chain of seven carbon atoms or less;

[0137] (vi) two of R_1—R_4 are CH_3, one of R_1—R_4 is $C_6H_5CH_2$, and one of R_1—R_4 is an alkyl chain of nineteen carbon atoms or more;

[0138] (vii) two of R_1—R_4 are CH_3 and one of R_1—R_4 is the group $C_6H_5(CH_2)_n$, where n>1;

[0139] (viii) two of R_1—R_4 are CH_3, one of R_1—R_4 is $C_6H_5CH_2$, and one of R_1—R_4 comprises at least one heteroatom;

[0140] (ix) two of R_1—R_4 are CH_3, one of R_1—R_4 is $C_6H_5CH_2$, and one of R_1—R_4 comprises at least one halogen;

[0141] (x) two of R_1—R_4 are CH_3, one of R_1—R_4 is $C_6H_5CH_2$, and one of R_1—R_4 comprises at least one cyclic fragment;

[0142] (xi) two of R_1—R_4 are CH_3 and one of R_1—R_4 is a phenyl ring; or

[0143] (xii) two of R_1—R_4 are CH_3 and two of R_1—R_4 are purely aliphatic fragments.

[0144] Such compounds include, but are not limited to, behenalkonium chloride, benzethonium chloride, cetylpyridinium chloride, behentrimonium chloride, lauralkonium chloride, cetalkonium chloride, cetrimonium bromide, cetrimonium chloride, cethylamine hydrofluoride, chlorallylmethenamine chloride (Quaternium-15), distearyldimonium chloride (Quaternium-5), dodecyl dimethyl ethylbenzyl ammonium chloride(Quaternium-14), Quaternium-22, Quaternium-26, Quaternium-18 hectorite, dimethylaminoethylchloride hydrochloride, cysteine hydrochloride, diethanolammonium POE (10) oletyl ether phosphate, diethanolammonium POE (3)oleyl ether phosphate, tallow alkonium chloride, dimethyl dioctadecylammoniumbentonite, stearalkonium chloride, domiphen bromide, denatonium benzoate, myristalkonium chloride, laurtrimonium chloride, ethylenediamine dihydrochloride, guanidine hydrochloride, pyridoxine HCl, infetamine hydrochloride, meglumine hydrochloride, methylbenzethonium chloride, myrtrimonium bromide, oleyltrimonium chloride, polyquaternium-1, procainehydrochloride, cocobetaine, stearalkonium bentonite, stearalkoniumhectonite, stearyl trihydroxyethyl propylenediamine dihydrofluoride, tallowtrimonium chloride, and hexadecyltrimethyl ammonium bromide.

[0145] In a preferred embodiment, the compositions of the invention do not contain as a surface stabilizer a polyalkylene block copolymer which gels at physiological temperature, and which contains one or more polyoxyethylene blocks and one or more polyoxy (higher alkylene) blocks, wherein at least some of the blocks are linked together by an oxymethylene group. Such compounds are described in U.S. Pat. No. 5,705,194.

[0146] Preferred surface stabilizers include, but are not limited to, Tween® 80, Tetronic® T908, dioctylsulfosuccinate, polyvinyl acetate, Pluronic® F127, or a mixture thereof.

[0147] Tween® 80 is polyoxyethylene sorbitan monooleate (polyoxyethylenesorbitan monooleat). Tetronic® T908 is a tetrafunctional block copolymer derived from sequential addition of ethylene oxide and propylene oxide to ethylene-diamine available from BASF. Pluronic® F127, which is a poloxamer, is a difunctional block copolymer surfactant of ethylene oxide and propylene oxide terminating in primary hydroxyl groups and having a molecular weight of about 12,600 daltons (see

[0148] http://www.basf.com/businesses/chemicals/performance/pdfs/Pluronic_F127.pdf. Pluronic® F127 has the chemical formula of $PEO_{100}PPO_{65}PEO_{100}$.

[0149] Most of these surface stabilizers are known pharmaceutical excipients and are described in detail in the *Handbook of pharmaceutical Excipients*, published jointly by the American Pharmaceutical Association and The Pharmaceutical Society of Great Britain (The Pharmaceutical Press, 2000), specifically incorporated by reference.

[0150] C. Pharmaceutical Excipients

[0151] Pharmaceutical compositions according to the invention may also comprise one or more binding agents, filling agents, lubricating agents, suspending agents, sweeteners, flavoring agents, preservatives, buffers, wetting agents, disintegrants, effervescent agents, and other excipients. Such excipients are known in the art.

[0152] Examples of filling agents are lactose monohydrate, lactose anhydrous, and various starches; examples of binding agents are various celluloses and cross-linked polyvinylpyrrolidone, microcrystalline cellulose, such as Avicel® PH101 and Avicel® PH102, microcrystalline cellulose, and silicified microcrystalline cellulose (ProSolv SMCC™).

[0153] Suitable lubricants, including agents that act on the flowability of the powder to be compressed, are colloidal silicon dioxide, such as Aerosil® 200, talc, stearic acid, magnesium stearate, calcium stearate, and silica gel.

[0154] Examples of sweeteners are any natural or artificial sweetener, such as sucrose, xylitol, sodium saccharin, cyclamate, aspartame, and acesulfame. Examples of flavoring agents are Magnasweet® (trademark of MAFCO), bubble gum flavor, and fruit flavors, and the like.

[0155] Examples of preservatives are potassium sorbate, methylparaben, propylparaben, benzoic acid and its salts, other esters of parahydroxybenzoic acid such as butylparaben, alcohols such as ethyl or benzyl alcohol, phenolic compounds such as phenol, or quarternary compounds such as benzalkonium chloride.

[0156] Suitable diluents include pharmaceutically acceptable inert fillers, such as microcrystalline cellulose, lactose, dibasic calcium phosphate, saccharides, and/or mixtures of any of the foregoing. Examples of diluents include microcrystalline cellulose, such as Avicel® PH101 and Avicel® PH102; lactose such as lactose monohydrate, lactose anhy-

drous, and Pharmatose® DCL21; dibasic calcium phosphate such as Emcompress®; mannitol; starch; sorbitol; sucrose; and glucose.

[0157] Suitable disintegrants include lightly crosslinked polyvinyl pyrrolidone, corn starch, potato starch, maize starch, and modified starches, croscarmellose sodium, crospovidone, sodium starch glycolate, and mixtures thereof.

[0158] Examples of effervescent agents are effervescent couples such as an organic acid and a carbonate or bicarbonate. Suitable organic acids include, for example, citric, tartaric, malic, fumaric, adipic, succinic, and alginic acids and anhydrides and acid salts. Suitable carbonates and bicarbonates include, for example, sodium carbonate, sodium bicarbonate, potassium carbonate, potassium bicarbonate, magnesium carbonate, sodium glycine carbonate, L-lysine carbonate, and arginine carbonate. Alternatively, only the sodium bicarbonate component of the effervescent couple may be present.

[0159] D. Nanoparticulate Griseofulvin Particle Size

[0160] As used herein, particle size is determined on the basis of the weight average particle size as measured by conventional particle size measuring techniques well known to those skilled in the art. Such techniques include, for example, sedimentation field flow fractionation, photon correlation spectroscopy, light scattering, and disk centrifugation.

[0161] The compositions of the invention comprise griseofulvin nanoparticles which have an effective average particle size of less than about 2000 nm (i.e., 2 microns), less than about 1900 nm, less than less than about 1800 nm, less than about 1700 nm, less than about 1600 nm, less than about 1500 nm, less than about 1400 nm, less than about 1300 nm, less than about 1200 nm, less than about 1100 nm, less than about 1000 nm, less than about 900 nm, less than about 800 nm, less than about 700 nm, less than about 600 nm, less than about 500 nm, less than about 400 nm, less than about 300 nm, less than about 250 nm, less than about 200 nm, less than about 150 nm, less than about 140 nm, less than about 130 nm, less than about 120 nm, less than about 110 nm, less than about 100 nm, less than about 90 nm, less than about 80 nm, less than about 70 nm, less than about 60 nm, or less than about 50 nm, when measured by the above-noted techniques.

[0162] By "an effective average particle size of less than about 2000 nm" it is meant that at least 50% of the nanoparticulate griseofulvin particles have a weight average particle size less than about 2000 nm, when measured by the above-noted techniques. In other embodiments of the invention, at least about 70%, at least about 90%, at least about 95%, or at least about 99% of the griseofulvin particles have a particle size less than the effective average, by weight, i.e., less than about 2000 nm, less than about 1900 nm, less than less than about 1800 nm, less than about 1700 nm, etc.

[0163] If the nanoparticulate griseofulvin composition is combined with a microparticulate griseofulvin or non-griseofulvin active agent composition, then such a composition is either solubilized or has an effective average particle size greater than about 2 microns. By "an effective average particle size of greater than about 2 microns" it is meant that at least 50% of the microparticulate griseofulvin or non-griseofulvin active agent particles have a particle size greater than about 2 microns, by weight, when measured by the above-noted techniques. In other embodiments of the invention, at least about 70%, at least about 90%, at least about 95%, or at least about 99%, by weight, of the microparticulate griseofulvin or non-griseofulvin active agent particles have a particle size greater than about 2 microns.

[0164] In the present invention, the value for D50 of a nanoparticulate griseofulvin composition is the particle size below which 50% of the griseofulvin particles fall, by weight. Similarly, D90 and D99 are the particle sizes below which 90% and 99%, respectively, of the griseofulvin particles fall, by weight.

[0165] E. Concentration of Nanoparticulate Griseofulvin and Surface Stabilizers

[0166] The relative amounts of griseofulvin and one or more surface stabilizers can vary widely. The optimal amount of the individual components can depend, for example, upon the hydrophilic lipophilic balance (HLB), melting point, and the surface tension of water solutions of the stabilizer, etc.

[0167] The concentration of griseofulvin can vary from about 99.5% to about 0.001%, from about 95% to about 0.1%, or from about 90% to about 0.5%, by weight, based on the total combined dry weight of the griseofulvin and at least one surface stabilizer, not including other excipients.

[0168] The concentration of the at least one surface stabilizer can vary from about 0.5% to about 99.999%, from about 5.0% to about 99.9%, or from about 10% to about 99.5%, by weight, based on the total combined dry weight of the griseofulvin and at least one surface stabilizer, not including other excipients.

[0169] III. Methods of Making Nanoparticulate Griseofulvin Formulations

[0170] The nanoparticulate griseofulvin compositions can be made using, for example, milling, homogenization, or precipitation techniques. Exemplary methods of making nanoparticulate compositions are described in the '684 patent. Methods of making nanoparticulate compositions are also described in U.S. Pat. No. 5,518,187 for "Method of Grinding Pharmaceutical Substances;" U.S. Pat. No. 5,718,388 for "Continuous Method of Grinding Pharmaceutical Substances;" U.S. Pat. No. 5,862,999 for "Method of Grinding Pharmaceutical Substances;" U.S. Pat. No. 5,665,331 for "Co-Microprecipitation of Nanoparticulate Pharmaceutical Agents with Crystal Growth Modifiers;" U.S. Pat. No. 5,662,883 for "Co-Microprecipitation of Nanoparticulate Pharmaceutical Agents with Crystal Growth Modifiers;" U.S. Pat. No. 5,560,932 for "Microprecipitation of Nanoparticulate Pharmaceutical Agents;" U.S. Pat. No 5,543,133 for "Process of Preparing X-Ray Contrast Compositions Containing Nanoparticles;" U.S. Pat. No. 5,534,270 for "Method of Preparing Stable Drug Nanoparticles;" U.S. Pat. No. 5,510,118 for "Process of Preparing Therapeutic Compositions Containing Nanoparticles;" and U.S. Pat. No. 5,470,583 for "Method of Preparing Nanoparticle Compositions Containing Charged Phospholipids to Reduce Aggregation," all of which are specifically incorporated by reference.

[0171] Following milling, homogenization, precipitation, etc., the resultant nanoparticulate griseofulvin composition

can be utilized in solid or liquid dosage formulations, such as controlled release formulations, solid dose fast melt formulations, aerosol formulations, nasal formulations, lyophilized formulations, tablets, capsules, solid lozenge, powders, creams, ointments, etc.

[0172] A. Milling to Obtain Nanoparticulate Griseofulvin Dispersions

[0173] Milling griseofulvin to obtain a nanoparticulate dispersion comprises dispersing griseofulvin particles in a liquid dispersion media in which griseofulvin is poorly soluble, followed by applying mechanical means in the presence of grinding media to reduce the particle size of griseofulvin to the desired effective average particle size. The dispersion media can be, for example, water, safflower oil, ethanol, t-butanol, glycerin, polyethylene glycol (PEG), hexane, or glycol.

[0174] The griseofulvin particles can be reduced in size in the presence of at least one surface stabilizer. Alternatively, the griseofulvin particles can be contacted with one or more surface stabilizers after attrition. Other compounds, such as a diluent, can be added to the griseofulvin/surface stabilizer composition during the size reduction process. Dispersions can be manufactured continuously or in a batch mode.

[0175] B. Precipitation to Obtain Nanoparticulate Griseofulvin Compositions

[0176] Another method of forming the desired nanoparticulate griseofulvin composition is by microprecipitation. This is a method of preparing stable dispersions of poorly soluble active agents in the presence of one or more surface stabilizers and one or more colloid stability enhancing surface active agents free of any trace toxic solvents or solubilized heavy metal impurities. Such a method comprises, for example: (1) dissolving griseofulvin in a suitable solvent; (2) adding the formulation from step (1) to a solution comprising at least one surface stabilizer; and (3) precipitating the formulation from step (2) using an appropriate non-solvent. The method can be followed by removal of any formed salt, if present, by dialysis or diafiltration and concentration of the dispersion by conventional means.

[0177] C. Homogenization to Obtain Nanoparticulate Griseofulvin Compositions

[0178] Exemplary homogenization methods of preparing active agent nanoparticulate compositions are described in U.S. Pat. No. 5,510,118, for "Process of Preparing Therapeutic Compositions Containing Nanoparticles."

[0179] Such a method comprises dispersing griseofulvin particles in a liquid dispersion media in which griseofulvin is poorly soluble, followed by subjecting the dispersion to homogenization to reduce the particle size of the griseofulvin to the desired effective average particle size. The dispersion media can be, for example, water, safflower oil, ethanol, t-butanol, glycerin, polyethylene glycol (PEG), hexane, or glycol.

[0180] The griseofulvin particles can be reduced in size in the presence of at least one surface stabilizer. Alternatively, the griseofulvin particles can be contacted with one or more surface stabilizers either before or after attrition. Other compounds, such as a diluent, can be added to the griseofulvin/surface stabilizer composition either before, during, or after the size reduction process. Dispersions can be manufactured continuously or in a batch mode.

[0181] IV. Methods of Using Nanoparticulate Griseofulvin Formulations

[0182] The method of the invention comprises administering to a subject an effective amount of a composition comprising nanoparticulate griseofulvin. The griseofulvin compositions of the present invention can be administered to a subject via any conventional means including, but not limited to, orally, rectally, ocularly, parenterally (e.g., intravenous, intramuscular, or subcutaneous), intracisternally, pulmonary, intravaginally, intraperitoneally, locally (e.g., powders, ointments or drops), or as a buccal or nasal spray. As used herein, the term "subject" is used to mean an animal, preferably a mammal, including a human or non-human. The terms patient and subject may be used interchangeably.

[0183] The griseofulvin compositions of the invention can be used to treat dermatophyte and ringworm infections of the scalp, hair, nails (fingernails and toenails), and skin. The griseofulvin compositions can be used to treat skin infections such as jock itch, athlete's foot, and ringworm. In particular, the grisenfulvin compositions of the invention can be used to treat *Tinea capitis* (ringworm of the scalp), *Tinea corporis* (ringworm of the body), *Tinea pedis* (athlete's foot), *Tinea unguium* (ringworm of the nails), *Tinea cruris* (ringworm of the thigh), and *Tinea barbae* (barber's itch). The griseofulvin compositions of the invention can also used to the following fungal infections of the hair, skin, and nails: *Trichophyton rubrum, Trichophyton tonsurans, Trichophyton mentagrophytes, Trichophyton interdigitalis, Trichophyton verrucosum, Trichophyton sulphureum, Trichophyton schoenleini, Trichophyton audouini, Trichophyton canis, Trichophyton gypseum, Trichophyton floccosum, Trichophyton megnini, Trichophyton gallinae,* and *Trichophyton crateriform.*

[0184] Compositions suitable for parenteral injection may comprise physiologically acceptable sterile aqueous or nonaqueous solutions, dispersions, suspensions or emulsions, and sterile powders for reconstitution into sterile injectable solutions or dispersions. Examples of suitable aqueous and nonaqueous carriers, diluents, solvents, or vehicles including water, ethanol, polyols (propyleneglycol, polyethyleneglycol, glycerol, and the like), suitable mixtures thereof, vegetable oils (such as olive oil) and injectable organic esters such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

[0185] The nanoparticulate compositions may also contain adjuvants such as preserving, wetting, emulsifying, and dispensing agents. Prevention of the growth of microorganisms can be ensured by various antibacterial and antifungal agents, such as parabens, chlorobutanol, phenol, sorbic acid, and the like. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like. Prolonged absorption of the injectable pharmaceutical form can be brought about by the use of agents delaying absorption, such as aluminum monostearate and gelatin.

[0186] Solid dosage forms for oral administration include, but are not limited to, powder aerosols, capsules, tablets,

pills, powders, and granules. In such solid dosage forms, the active agent is admixed with at least one of the following: (a) one or more inert excipients (or carriers), such as sodium citrate or dicalcium phosphate; (b) fillers or extenders, such as starches, lactose, sucrose, glucose, mannitol, and silicic acid; (c) binders, such as carboxymethylcellulose, alignates, gelatin, polyvioylpyrrolidone, sucrose, and acacia; (d) humectants, such as glycerol; (e) disintegrating agents, such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain complex silicates, and sodium carbonate; (f) solution retarders, such as paraffin; (g) absorption accelerators, such as quaternary ammonium compounds; (h) wetting agents, such as cetyl alcohol and glycerol monostearate; (i) adsorbents, such as kaolin and bentonite; and (j) lubricants, such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, or mixtures thereof. For capsules, tablets, and pills, the dosage forms may also comprise buffering agents.

[0187] Liquid dosage forms for oral administration include pharmaceutically acceptable aerosols, emulsions, solutions, suspensions, syrups, and elixirs. In addition to the active agent, the liquid dosage forms may comprise inert diluents commonly used in the art, such as water or other solvents, solubilizing agents, and emulsifiers. Exemplary emulsifiers are ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propyleneglycol, 1,3-butyleneglycol, dimethylformamide, oils, such as cottonseed oil, groundnut oil, corn germ oil, olive oil, castor oil, and sesame oil, glycerol, tetrahydrofurfuryl alcohol, polyethyleneglycols, fatty acid esters of sorbitan, or mixtures of these substances, and the like.

[0188] Besides such inert diluents, the composition can also include adjuvants, such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, and perfuming agents.

[0189] One of ordinary skill will appreciate that effective amounts of griseofulvin can be determined empirically and can be employed in pure form or, where such forms exist, in pharmaceutically acceptable salt, ester, or prodrug form. Actual dosage levels of griseofulvin in the nanoparticulate compositions of the invention may be varied to obtain an amount of griseofulvin that is effective to obtain a desired therapeutic response for a particular composition and method of administration. The selected dosage level therefore depends upon the desired therapeutic effect, the route of administration, the potency of the administered griseofulvin, the desired duration of treatment, and other factors.

[0190] Dosage unit compositions may contain such amounts of such submultiples thereof as may be used to make up the daily dose. It will be understood, however, that the specific dose level for any particular patient will depend upon a variety of factors: the type and degree of the cellular or physiological response to be achieved; activity of the specific agent or composition employed; the specific agents or composition employed; the age, body weight, general health, sex, and diet of the patient; the time of administration, route of administration, and rate of excretion of the agent; the duration of the treatment; drugs used in combination or coincidental with the specific agent; and like factors well known in the medical arts.

[0191] The following examples are given to illustrate the present invention. It should be understood, however, that the invention is not to be limited to the specific conditions or details described in these examples. Throughout the specification, any and all references to a publicly available document, including a U.S. patent, are specifically incorporated by reference.

EXAMPLE 1

[0192] The purpose of this example was to prepare a nanoparticulate griseofulvin composition.

[0193] 5.0% (w/w) griseofulvin and 2.5% (w/w) Pluronic® F68 were combined in an aqueous media (water). 3.75 mLs of this mixture was then charged into a ½ oz bottle (15 ml) for roller milling on a Bench top roller mill (U.S. Stoneware, East Palestine, Ohio) along with 1.0 mm zirconium oxide milling media. The griseofulvin slurry was then milled for 2 days.

[0194] Following milling, the D50 particle size of the griseofulvin particles was 617 nm, and the D90 was 1000 nm. Particle size was determined on the Coulter Model N4MD Submicron Particle Analyzer (Coulter Corp., Miami Lakes, Fla.), and using the Microtrac Ultrafine Particle Analyzer (Leeds and Northrup Co., St. Petersburg, Fla.).

[0195] This example demonstrates that nanoparticulate compositions of griseofulvin can be made.

EXAMPLE 2

[0196] The purpose of this example was to prepare a nanoparticulate griseofulvin composition.

[0197] 5.0% (w/w) griseofulvin and 2.5% (w/w) Pluronic® 127 were combined in an aqueous media (water). 3.75 mLs of this mixture was then charged into a ½ oz bottle (15 mL) for roller milling on a Bench top roller mill (U.S. Stoneware, East Palestine, Ohio) along with 1.0 mm zirconium oxide milling media. The griseofulvin slurry was then milled for 5 days.

[0198] Following milling, the D90 particle size of the griseofulvin particles was 464 nm. Particle size was determined on the Coulter Model N4MD Submicron Particle Analyzer (Coulter Corp., Miami Lakes, Fla.), and using the Microtrac Ultrafine Particle Analyzer (Leeds and Northrup Co., St. Petersburg, Fla.).

[0199] This example demonstrates that nanoparticulate compositions of griseofulvin can be made.

EXAMPLE 3

[0200] The purpose of this example was to prepare a pharmaceutical composition utilizing the nanoparticulate griseofulvin composition of Example 2.

[0201] The nanoparticulate griseofulvin composition of Example 2 was combined with pharmaceutical excipients and carriers as shown below in Table 1.

TABLE 1

Ingredient	Quantity
Griseofulvin	5.0 g
Pluronic F127	2.5 g
Benzoate Sodium	0.2 g
Saccharin Sodium	0.1 g

TABLE 1-continued

Ingredient	Quantity
FD&C Red No. 3	0.03 g
Water, qs	100 mL

[0202] This example demonstrates the successful preparation of a pharmaceutical composition comprising a nanoparticulate griseofulvin composition.

EXAMPLE 4

[0203] The purpose of this example was to prepare nanoparticulate griseofulvin compositions using various surface stabilizers.

[0204] An aqueous slurry of 5% (w/w) griseofulvin and 2.5% surface stabilizer(s) in water was prepared. The surface stabilizer(s) are identified in Table 2, below. 3.75 mLs of each slurry was then charged into a ½ oz bottle (15 mL) for roller milling on a Bench top roller mill (U.S. Stoneware, East Palestine, Ohio) along with 1.0 mm zirconium oxide milling media. Each slurry was then milled for the time period shown in Table 2.

[0205] Following completion of milling, the average particle size, by weight, of the griseofulvin was determined. In addition, the D80 and/or D90 griseofulvin particle sizes were determined. Particle size was determined on the Coulter Model N4MD Submicron Particle Analyzer (Coulter Corp., Miami Lakes, Fla.), and using the Microtrac Ultrafine Particle Analyzer (Leeds and Northrup Co., St. Petersburg, Fla.).

TABLE 2

Surface Stabilizer and Quantity Thereof	Milling Time (days)	Weight Average Griseofulvin Particle Size (D50) (nm)	D80 (nm)	D90 (nm)
2.5% polyvinyl acetate 70–100K (PVA)	2	459		464
2.5% PVA 70–100K + 2.5% dioctylsulfosuccinate (DOSS)	10	276	215	464
2.5% DOSS	2	225		215
2.5% DOSS 2.5% DOSS	10	224	215	464
2.5% Tetronic ® 908	2	101		100
2.5% Tetronic ® 908 + 2.5% DOSS	10	310		464
2.5% Pluronic ® F127	5	519		464
2.5% Pluronic ® F127 + 2.5% DOSS	10	309		2150
2.5% tyloxapol	5	492	215	464
2.5% tyloxapol + 2.5% DOSS	6	279	215	464
2.5% Tween ® 80	6	290	215	464
2.5% Tween ® 80 + 2.5% DOSS	6	282	215	464
2.5% Pharmacoat ® 603	6	77.5		10,000
2.5% Pharmacoat ® 603 + 2.5% DOSS	2	438	464	1000
2.5% Pluronic ® F68	6	617		1000
2.5% Pluronic ® F68 + 2.5% DOSS	2	864		10,000
2.5% Polyvinylpyrrolidone K15 (PVP)	2	455		10,000

TABLE 2-continued

Surface Stabilizer and Quantity Thereof	Milling Time (days)	Weight Average Griseofulvin Particle Size (D50) (nm)	D80 (nm)	D90 (nm)
2.5% PVP K15 + 2.5% DOSS	10	270	215	464

[0206] All of the milled compositions were tested for stability in water, simulated gastric fluid (SGF), and simulated intestinal fluid (SIF) (SGF and SIF were prepared according to the USP). In addition, the stability of the milled compositions following storage for one week at room temperature was determined.

[0207] The particle morphology of griseofulvin appears to be spherical in nature when observed by light microscopy.

[0208] Based on the above data, and the lack of aggregation in fluid stability tests and stable one week shelf stabilities, the following surface stabilizers for Nanoparticulate griseofulvin are preferred:

[0209] (1) Tween® 80: This surface stabilizer is Generally Recognized as Safe (GRAS) (see "Inactive Ingredient Guide, Division of Drug Information Resources Food and Drug Administration, Center for Drug Evaluation and Research, Office of Management, January 1996). In addition, nanoparticulate griseofulvin compositions utilizing this surface stabilizer exhibited small size particles and were stable in water, simulated gastric fluid, and simulated intestinal fluid;

[0210] (2) Tetronic® T908: This surface stabilizer is GRAS, and nanoparticulate griseofulvin compositions utilizing this surface stabilizer exhibited small size particles and were stable in water, simulated gastric fluid, and simulated intestinal fluid; and

[0211] (3) Dioctylsulfosuccinate: This surface stabilizer is GRAS, and nanoparticulate griseofulvin compositions utilizing this surface stabilizer exhibited small size particles and were stable in water, simulated gastric fluid, and simulated intestinal fluid.

[0212] Less preferred but still useful are nanoparticulate griseofulvin compositions comprising as a surface stabilizer polyvinyl acetate or Pluronic® 127.

[0213] The following surface stabilizers performed poorly during the milling assay, and exhibited poor stability in water, simulated gastric fluid, and/or simulated intestinal fluid:

[0214] (1) Pharmacoat® 603: A bimodal griseofulvin particle size distribution and large griseofulvin particles were observed;

[0215] (2) Pluronic(® F68: A bimodal griseofulvin particle size distribution was observed;

[0216] (3) Ponlyvinylpyrrolidone: The griseofulvin composition comprising PVP as a surface stabilizer exhibited poor stability in water, simulated gastric fluid, and simulated intestinal fluid; and

[0217] (4) Tyloxapol: The griseofulvin composition comprising tyloxapol as a surface stabilizer exhibited poor stability in water, simulated gastric fluid, and simulated intestinal fluid.

[0218] While the latter four surface stabilizers were not successful in producing a stable nanoparticulate griseofulvin composition in this experiment, the surface stabilizers may be useful at different concentrations of griseofulvin or surface stabilizer, or when used in combination with one or more other surface stabilizers.

[0219] It will be apparent to those skilled in the art that various modifications and variations can be made in the methods and compositions of the present invention without departing from the spirit or scope of the invention. Thus, it is intended that the present invention cover the modifications and variations of this invention provided they come within the scope of the appended claims and their equivalents.

We claim:

1. A composition comprising:

(a) particles of griseofulvin or a salt thereof, wherein the griseofulvin particles have an effective average particle size of less than about 2000 nm; and

(b) at least one surface stabilizer, wherein the surface stabilizer is not a polyalkylene block copolymer which gels at physiological temperature, and which contains one or more polyoxyethylene blocks and one or more polyoxy (higher alkylene) blocks, wherein at least some of the blocks are linked together by an oxymethylene group.

2. The composition of claim 1, wherein the griseofulvin is selected from the group consisting of a crystalline phase, an amorphous phase, a semi-crystalline phase, a semi-amorphous phase, and mixtures thereof.

3. The composition of claim 1, wherein the effective average particle size of the griseofulvin particles is selected from the group consisting of less than about 1900 nm, less than about 1800 nm, less than about 1700 nm, less than about 1600 nm, less than about 1500 nm, less than about 1400 nm, less than about 1300 nm, less than about 1200 nm, less than about 1100 nm, less than about 1000 nm, less than about 900 nm, less than about 800 nm, less than about 700 nm, less than about 600 nm, less than about 500 nm, less than about 400 nm, less than about 300 nm, less than about 250 nm, less than about 200 nm, less than about 100 nm, less than about 75 nm, and less than about 50 nm.

4. The composition of claim 1, wherein the composition is formulated for administration selected from the group consisting of oral, pulmonary, rectal, opthalmic, colonic, parenteral, intracisternal, intravaginal, intraperitoneal, local, buccal, nasal, and topical administration.

5. The composition of claim 1 formulated into a dosage form selected from the group consisting of liquid dispersions, oral suspensions, gels, aerosols, ointments, creams, tablets, capsules, sachets, lozenges, powders, pills, and granules.

6. The composition of claim 1 formulated into a dosage form selected from the group consisting of controlled release formulations, fast melt formulations, lyophilized formulations, delayed release formulations, extended release formulations, pulsatile release formulations, and mixed immediate release and controlled release formulations.

7. The composition of claim 1, wherein the composition further comprises one or more pharmaceutically acceptable excipients, carriers, or a combination thereof.

8. The composition of claim 1, wherein griseofulvin or a salt thereof is present in an amount selected from the group consisting of from about 99.5% to about 0.001%, from about 95% to about 0.1%, and from about 90% to about 0.5%, by weight, based on the total combined dry weight of the griseofulvin or a salt thereof and at least one surface stabilizer, not including other excipients.

9. The composition of claim 1, wherein the at least one surface stabilizer is present in an amount selected from the group consisting of from about 0.5% to about 99.999% by weight, from about 5.0% to about 99.9% by weight, and from about 10% to about 99.5% by weight, based on the total combined dry weight of the griseofulvin or a salt thereof and at least one surface stabilizer, not including other excipients.

10. The composition of claim 1, comprising at least two surface stabilizers.

11. The composition of claim 1, wherein the surface stabilizer is selected from the group consisting of an anionic surface stabilizer, a cationic surface stabilizer, a zwitterionic surface stabilizer, and an ionic surface stabilizer.

12. The composition of claim 11, wherein the at least one surface stabilizer is selected from the group consisting of cetyl pyridinium chloride, gelatin, casein, phosphatides, dextran, glycerol, gum acacia, cholesterol, tragacanth, stearic acid, benzalkonium chloride, calcium stearate, glycerol monostearate, cetostearyl alcohol, cetomacrogol emulsifying wax, sorbitan esters, polyoxyethylene alkyl ethers, polyoxyethylene castor oil derivatives, polyoxyethylene sorbitan fatty acid esters, polyethylene glycols, dodecyl trimethyl ammonium bromide, polyoxyethylene stearates, colloidal silicon dioxide, phosphates, sodium dodecylsulfate, carboxymethylcellulose calcium, hydroxypropyl celluloses, hypromellose, carboxymethylcellulose sodium, methylcellulose, hydroxyethylcellulose, hypromellose phthalate, noncrystalline cellulose, magnesium aluminum silicate, triethanolamine, polyvinyl alcohol, polyvinylpyrrolidone, 4-(1,1,3,3-tetramethylbutyl)-phenol polymer with ethylene oxide and formaldehyde, poloxamers; poloxamines, a charged phospholipid, dioctylsulfosuccinate, dialkylesters of sodium sulfosuccinic acid, sodium lauryl sulfate, alkyl aryl polyether sulfonates, mixtures of sucrose stearate and sucrose distearate, p-isononylphenoxypoly-(glycidol), decanoyl-N-methylglucamide; n-decyl β-D-glucopyranoside; n-decyl ,β-D-maltopyranoside; n-dodecyl β-D-glucopyranoside; n-dodecyl ,β-D-maltoside; heptanoyl-N-methylglucamide; n-heptyl-β-D-glucopyranoside; n-heptyl β-D-thioglucoside; n-hexyl ,β-D-glucopyranoside; nonanoyl-N-methylglucamide; n-noyl β-D-glucopyranoside; octanoyl-N-methylglucamide; n-octyl-β-D-glucopyranoside; octyl ,β-D-thioglucopyranoside; lysozyme, PEG-phospholipid, PEG-cholesterol, PEG-cholesterol derivative, PEG-vitamin A, and random copolymers of vinyl acetate and vinyl pyrrolidone.

13. The composition of claim 11, wherein the at least one cationic surface stabilizer is selected from the group consisting of a polymer, a biopolymer, a polysaccharide, a cellulosic, an alginate, a nonpolymeric compound, and a phospholipid.

14. The composition of claim 11, wherein the surface stabilizer is selected from the group consisting of cationic

lipids, polymethylmethacrylate trimethylammonium bromide, sulfonium compounds, polyvinylpyrrolidone-2-dimethylaminoethyl methacrylate dimethyl sulfate, hexadecyltrimethyl ammonium bromide, phosphonium compounds, quaternary ammonium compounds, benzyl-di(2-chloroethyl)ethylammonium bromide, coconut trimethyl ammonium chloride, coconut trimethyl ammonium bromide, coconut methyl dihydroxyethyl ammonium chloride, coconut methyl dihydroxyethyl ammonium bromide, decyl triethyl ammonium chloride, decyl dimethyl hydroxyethyl ammonium chloride, decyl dimethyl hydroxyethyl ammonium chloride bromide, C_{12-15} dimethyl hydroxyethyl ammonium chloride, C_{12-15} dimethyl hydroxyethyl ammonium chloride bromide, coconut dimethyl hydroxyethyl ammonium chloride, coconut dimethyl hydroxyethyl ammonium bromide, myristyl trimethyl ammonium methyl sulphate, lauryl dimethyl benzyl ammonium chloride, lauryl dimethyl benzyl ammonium bromide, lauryl dimethyl(ethenoxy)$_4$ ammonium chloride, lauryl dimethyl(ethenoxy)$_4$ ammonium bromide, N-alkyl(C_{12-18})dimethylbenzyl ammonium chloride, N-alkyl(C_{14-18})dimethyl-benzyl ammonium chloride, N-tetradecylidmethylbenzyl ammonium chloride monobydrate, dimethyl didecyl ammonium chloride, N-alkyl and (C_{12-14})dimethyl 1-naphthylmethyl ammonium chloride, trimethylammonium halide, alkyl-trimethylammonium salts, dialkyl-dimethylammonium salts, lauryl trimethyl ammonium chloride, ethoxylated alkyamidoalkyldialkylammonium salt, an ethoxylated trialkyl ammonium salt, dialkylbenzene dialkylammonium chloride, N-didecyldimethyl ammonium chloride, N-tetradecyldimethylbenzyl ammonium, chloride monohydrate, N-alkyl (C_{12-14})dimetbyl 1-naphthylmethyl ammonium chloride, dodecyldimethylbenzyl ammonium chloride, dialkyl benzenealkyl ammonium chloride, lauryl trimethyl ammonium chloride, alkylbenzyl methyl ammonium chloride, alkyl benzyl dimethyl ammonium bromide, C_{12} trimethyl ammonium bromides, C_{15} trimethyl ammonium bromides, C_{17} trimethyl ammonium bromides, dodecylbenzyl triethyl ammonium chloride, poly-diallyldimethylammonium chloride (DADMAC), dimethyl ammonium chlorides, alkyldimethylammonium halogenides, tricetyl methyl ammonium chloride, decyltrimethylammonium bromide, dodecyltriethylammonium bromide, tetradecyltrimethylammonium bromide, methyl trioctylammonium chloride, POLYQUAT 10™, tetrabutylammonium bromide, benzyl trimethylammonium bromide, choline esters, benzalkonium chloride, stearalkonium chloride compounds, cetyl pyridinium bromide, cetyl pyridinium chloride, halide salts of quaternized polyoxyethylalkylamines, MIRAPOL™, ALKAQUAT™, alkyl pyridinium salts; amines, amine salts, amine oxides, imide azolinium salts, protonated quaternary acrylamides, methylated quaternary polymers, and cationic guar.

15. The composition of any of claims **11**, **13**, or **14**, wherein the composition is bioadhesive.

16. The composition of claim 1, comprising as a surface stabilizer Tween(& 80, Tetronic® T908, dioctylsulfosuccinate, polyvinyl acetate, Pluronic® F127, or a mixture thereof.

17. The composition of claim 1, further comprising at least one additional griseofulvin composition having an effective average particle size which is different that the effective average particle size of the griseofulvin composition of claim 1

18. The composition of claim 1, additionally comprising one or more non-griseofulvin active agents.

19. The composition of claim 18, wherein said additional one or more non-griseofulvin active agents are selected from the group consisting of nutraceuticals, amino acids, proteins, peptides, nucleotides, anti-obesity drugs, central nervous system stimulants, carotenoids, corticosteroids, elastase inhibitors, anti-fungals, oncology therapies, anti-emetics, analgesics, cardiovascular agents, anti-inflammatory agents, antihelmintics, anti-arrhythmic agents, antibiotics, anticoagulants, antidepressants, antidiabetic agents, antiepileptics, antihistamines, antihypertensive agents, antimuscarinic agents, antimycobacterial agents, antineoplastic agents, immunosuppressants, antithyroid agents, antiviral agents, anxiolytics, sedatives, astringents, alpha-adrenergic receptor blocking agents, beta-adrenoceptor blocking agents, blood products, blood substitutes, cardiac inotropic agents, contrast media, corticosteroids, cough suppressants, diagnostic agents, diagnostic imaging agents, diuretics, dopaminergics, haemostatics, immunological agents, lipid regulating agents, muscle relaxants, parasympathomimetics, parathyroid calcitonin, parathyroid biphosphonates, prostaglandins, radiopharmaceuticals, sex hormones, anti-allergic agents, stimulants, anoretics, sympathomimetics, thyroid agents, vasodilators, and xanthines.

20. The composition of claim 18, wherein said at least one additional non-griseofulvin active agent is an anti-fungal agent.

21. The composition of claim 18, wherein said at least one additional non-griseofulvin active agent is vitamin E.

22. The composition of claim 1, wherein upon administration to a mammal the griseofulvin particles redisperse such that the particles have an effective average particle size of less than about 2 microns.

23. The composition of claim 22, wherein upon administration the composition redisperses such that the griseofulvin particles have an effective average particle size selected from the group consisting of less than about 1900 nm, less than about 1800 nm, less than about 1700 nm, less than about 1600 nm, less than about 1500 nm, less than about 1400 nm, less than about 1300 nm, less than about 1200 nm, less than about 1100 nm, less than about 1000 nm, less than about 900 nm, less than about 800 nm, less than about 700 nm, less than about 600 nm, less than about 500 nm, less than about 400 nm, less than about 300 nm, less than about 250 nm, less than about 200 nm, less than about 150 nm, less than about 100 nm, less than about 75 nm, and less than about 50 nm.

24. The composition of claim 1, wherein the composition redisperses in a biorelevant media such that the griseofulvin particles have an effective average particle size of less than about 2 microns.

25. The composition of claim 24, wherein the biorelevant media is selected from the group consisting of water, aqueous electrolyte solutions, aqueous solutions of a salt, aqueous solutions of an acid, aqueous solutions of a base, and combinations thereof.

26. The composition of claim 24, wherein the composition redisperses in a biorelevant media such that the griseofulvin particles have an effective average particle size selected from the group consisting of less than about 1900 nm, less than about 1800 nm, less than about 1700 nm, less than about 1600 nm, less than about 1500 nm, less than about 1400 nm, less than about 1300 nm, less than about

1200 nm, less than about 1100 nm, less than about 1000 nm, less than about 900 nm, less than about 800 nm, less than about 700 nm, less than about 600 nm, less than about 500 nm, less than about 400 nm, less than about 300 nm, less than about 250 nm, less than about 200 nm, less than about 150 nm, less than about 100 nm, less than about 75 nm, and less than about 50 nm.

27. The composition of claim 1, wherein the T_{max} of the griseofulvin, when assayed in the plasma of a mammalian subject following administration, is less than the T_{max} for a non-nanoparticulate griseofulvin formulation, administered at the same dosage.

28. The composition of claim 27, wherein the T_{max} is selected from the group consisting of not greater than about 90%, not greater than about 80%, not greater than about 70%, not greater than about 60%, not greater than about 50%, not greater than about 30%, not greater than about 25%, not greater than about 20%, not greater than about 15%, not greater than about 10%, and not greater than about 5% of the T_{max} exhibited by a non-nanoparticulate griseofulvin formulation, administered at the same dosage.

29. The composition of claim 1, wherein the C_{max} of the griseofulvin, when assayed in the plasma of a mammalian subject following administration, is greater than the C_{max} for a non-nanoparticulate griseofulvin formulation, administered at the same dosage.

30. The composition of claim 29, wherein the C_{max} is selected from the group consisting of at least about 50%, at least about 100%, at least about 200%, at least about 300%, at least about 400%, at least about 500%, at least about 600%, at least about 700%, at least about 800%, at least about 900%, at least about 1000%, at least about 1100%, at least about 1200%, at least about 1300%, at least about 1400%, at least about 1500%, at least about 1600%, at least about 1700%, at least about 1800%, or at least about 1900% greater than the C_{max} exhibited by a non-nanoparticulate formulation of griseofulvin, administered at the same dosage.

31. The composition of claim 1, wherein the AUC of the griseofulvin, when assayed in the plasma of a mammalian subject following administration, is greater than the AUC for a non-nanoparticulate griseofulvin formulation, administered at the same dosage.

32. The composition of claim 31, wherein the AUC is selected from the group consisting of at least about 25%, at least about 50%, at least about 75%, at least about 100%, at least about 125%, at least about 150%, at least about 175%, at least about 200%, at least about 225%, at least about 250%, at least about 275%, at least about 300%, at least about 350%, at least about 400%, at least about 450%, at least about 500%, at least about 550%, at least about 600%, at least about 750%, at least about 700%, at least about 750%, at least about 800%, at least about 850%, at least about 900%, at least about 950%, at least about 1000%, at least about 1050%, at least about 1100%, at least about 1150%, or at least about 1200% greater than the AUC exhibited by the non-nanoparticulate formulation of griseofulvin, administered at the same dosage.

33. The composition of claim 1 which does not produce significantly different absorption levels when administered under fed as compared to fasting conditions.

34. The composition of claim 33, wherein the difference in absorption of the griseofulvin composition of the invention, when administered in the fed versus the fasted state, is selected from the group consisting of less than about 100%, less than about 90%, less than about 80%, less than about 70%, less than about 60%, less than about 50%, less than about 40%, less than about 30%, less than about 25%, less than about 20%, less than about 15%, less than about 10%, less than about 5%, and less than about 3%.

35. The composition of claim 1, wherein administration of the composition to a human in a fasted state is bioequivalent to administration of the composition to a subject in a fed state.

36. The composition of claim 35, wherein "bioequivalency" is established by a 90% Confidence Interval of between 0.80 and 1.25 for both C_{max} and AUC.

37. The composition of claim 35, wherein "bioequivalency" is established by a 90% Confidence Interval of between 0.80 and 1.25 for AUC and a 90% Confidence Interval of between 0.70 to 1.43 for C_{max}.

38. The composition of claim 1 formulated into a liquid dosage form, wherein the dosage form has a viscosity of less than about 2000 mPa.s, measured at 20° C., at a shear rate of 0.1 (1/s).

39. The composition of claim 38, having a viscosity at a shear rate of 0.1 (1/s), measured at 20° C., selected from the group consisting of from about 2000 mPa.s to about 1 mpa.s, from about 1900 mPa.s to about 1 mPa.s, from about 1800 mPa.s to about 1 mPa.s, from about 1700 mPa.s to about 1 mPa.s, from about 1600 mPa.s to about 1 mPa.s, from about 1500 mPa.s to about 1 mPa.s, from about 1400 mPa.s to about 1 mPa.s, from about 1300 mPa.s to about 1 mPa.s, from about 1200 mPa.s to about 1 mPa.s, from about 1100 mPa.s to about 1 mPa.s, from about 1000 mpPs to about 1 mPa.s, from about 900 mPa.s to about 1 mPa.s, from about 800 mPa.s to about 1 mPa.s, from about 700 mPa.s to about 1 mPa.s, from about 600 mPa.s to about 1 mPa.s, from about 500 mPa.s to about 1 mPa.s, from about 400 mPa.s to about 1 mPa.s, from about 300 mPa.s to about 1 mPa.s, from about 200 mPa.s to about 1 mPa.s, from about 175 mPa.s to about 1 mPa.s, from about 150 mPa.s to about 1 mPa.s, from about 125 mPa.s to about 1 mPa.s , from about 100 mPa.s to about 1 mPa.s, from about 75 mPa.s to about 1 mPa.s , from about 50 mPa.s to about 1 mPa.s , from about 25 mPa.s to about 1 mPa.s , from about 15 mPa.s to about 1 mPa.s , from about 10 mPa.s to about 1 mPa.s, and from about S mPa.s to about 1 mPa.s .

40. The composition of claim 38, wherein the viscosity of the dosage form is selected from the group consisting of less than about 1/200, less than about 1/100, less than about 1/50, less than about 1/25, and less than about 1/10 of the viscosity of a liquid dosage form of a non-nanoparticulate composition of griseofulvin, at about the same concentration per ml of griseofulvin.

41. The composition of claim 38, wherein the viscosity of the dosage form is selected from the group consisting of less than about 5%, less than about 10%, less than about 15%, less than about 20%, less than about 25%, less than about 30%, less than about 35%, less than about 40%, less than about 45%, less than about 50%, less than about 55%, less than about 60%, less than about 65%, less than about 70%, less than about 75%, less than about 80%, less than about 85%, and less than about 90% of the viscosity of a liquid dosage form of a non-nanoparticulate composition of griseofulvin, at about the same concentration per ml of griseofulvin.

42. A method of making a griseofulvin composition comprising contacting particles of griseofulvin or a salt thereof with at least one surface stabilizer for a time and under conditions sufficient to provide a griseofulvin composition having an effective average particle size of less than about 2000 nm, wherein the surface stabilizer is not a polyalkylene block copolymer which gels at physiological temperature, and which contains one or more polyoxyethylene blocks and one or more polyoxy (higher alkylene) blocks, wherein at least some of the blocks are linked together by an oxymethylene group.

43. The method of claim 42, wherein said contacting comprises grinding.

44. The method of claim 43, wherein said grinding comprises wet grinding.

45. The method of claim 42, wherein said contacting comprises homogenizing.

46. The method of claim 42, wherein said contacting comprises:

(a) dissolving the particles of a griseofulvin or a salt thereof in a solvent;

(b) adding the resulting griseofulvin solution to a solution comprising at least one surface stabilizer; and

(c) precipitating the solubilized griseofulvin having at least one surface stabilizer adsorbed on the surface thereof by the addition thereto of a non-solvent.

47. The method of claim 42, wherein the griseofulvin or a salt thereof is selected from the group consisting of a crystalline phase, an amorphous phase, a semi-crystalline phase, a semi-amorphous phase, and mixtures thereof.

48. The method of claim 42, wherein the effective average particle size of the griseofulvin particles is selected from the group consisting of less than about 1900 nm, less than about 1800 nm, less than about 1700 nm, less than about 1600 nm, less than about 1500 nm, less than about 1000 nm, less than about 1400 nm, less than about 1300 nm, less than about 1200 nm, less than about 1100 nm, less than about 900 nm, less than about 800 nm, less than about 700 nm, less than about 600 nm, less than about 500 nm, less than about 400 nm, less than about 300 nm, less than about 250 nm, less than about 200 nm, less than about 100 nm, less than about 75 nm, and less than about 50 nm.

49. The method of claim 42, wherein the composition is formulated for administration selected from the group consisting of oral, pulmonary, rectal, opthalmic, colonic, parenteral, intracistemal, intravaginal, intraperitoneal, local, buccal, nasal, and topical administration.

50. The method of claim 42, wherein the composition further comprises one or more pharmaceutically acceptable excipients, carriers, or a combination thereof.

51. The method of claim 42, wherein griseofulvin or a salt thereof is present in an amount selected from the group consisting of from about 99.5% to about 0.001%, from about 95% to about 0.1%, and from about 90% to about 0.5%, by weight, based on the total combined dry weight of the griseofulvin or a salt thereof and at least one surface stabilizer, not including other excipients.

52. The method of claim 42, wherein the at least one surface stabilizer is present in an amount selected from the group consisting of from about 0.5% to about 99.999%, from about 5.0% to about 99.9%, and from about 10% to about 99.5% by weight, based on the total combined dry weight of the griseofulvin or a salt thereof and at least one surface stabilizer, not including other excipients.

53. The method of claim 42, utilizing at least two surface stabilizers.

54. The method of claim 42, wherein the surface stabilizer is selected from the group consisting of an anionic surface stabilizer, a cationic surface stabilizer, a zwitterionic surface stabilizer, and an ionic surface stabilizer.

55. The method of claim 54, wherein the at least one surface stabilizer is selected from the group consisting of cetyl pyridinium chloride, gelatin, casein, phosphatides, dextran, glycerol, gum acacia, cholesterol, tragacanth, stearic acid, benzalkonium chloride, calcium stearate, glycerol monostearate, cetostearyl alcohol, cetomacrogol emulsifying wax, sorbitan esters, polyoxyethylene alkyl ethers, polyoxyethylene castor oil derivatives, polyoxyethylene sorbitan fatty acid esters, polyethylene glycols, dodecyl trimethyl ammonium bromide, polyoxyethylene stearates, colloidal silicon dioxide, phosphates, sodium dodecylsulfate, carboxymethylcellulose calcium, hydroxypropyl celluloses, hypromellose, carboxymethylcellulose sodium, methylcellulose, hydroxyethylcellulose, hypromellose phthalate, noncrystalline cellulose, magnesium aluminum silicate, triethanolamine, polyvinyl alcohol, polyvinylpyrrolidone, 4-(1,1,3,3-tetramethylbutyl)-phenol polymer with ethylene oxide and formaldehyde, poloxamers; poloxamines, a charged phospholipid, dioctylsulfosuccinate, dialkylesters of sodium sulfosuccinic acid, sodium lauryl sulfate, alkyl aryl polyether sulfonates, mixtures of sucrose stearate and sucrose distearate, p-isononylphenoxypoly-(glycidol), decanoyl-N-methylglucamide; n-decyl β-D-glucopyranoside; n-decyl β-D-maltopyranoside; n-dodecyl β-D-glucopyranoside; n-dodecyl β-D-maltoside; heptanoyl-N-methylglucamide; n-heptyl-β-D-glucopyranoside; n-heptyl β-D-thioglucoside; n-hexyl β-D-glucopyranoside; nonanoyl-N-methylglucamide; n-noyl β-D-glucopyranoside; octanoyl-N-methylglucamide; n-octyl-β-D-glucopyranoside; octyl β-D-thioglucopyranoside; lysozyme, PEG-phospholipid, PEG-cholesterol, PEG-cholesterol derivative, PEG-vitamin A, PEG-vitamin E, and random copolymers of vinyl acetate and vinyl pyrrolidone.

56. The method of claim 54, wherein the at least one cationic surface stabilizer is selected from the group consisting of a polymer, a biopolymer, a polysaccharide, a cellulosic, an alginate, a nonpolymeric compound, and a phospholipid.

57. The method of claim 54, wherein the surface stabilizer is selected from the group consisting of cationic lipids, polymethylmethacrylate trimethylammonium bromide, sulfonium compounds, polyvinylpyrrolidone-2-dimethylaminoethyl methacrylate dimethyl sulfate, hexadecyltrimethyl ammonium bromide, phosphonium compounds, quarternary ammonium compounds, benzyl-di(2-chloroethyl)ethylammonium bromide, coconut trimethyl ammonium chloride, coconut trimethyl ammonium bromide, coconut methyl dihydroxyethyl ammonium chloride, coconut methyl dihydroxyethyl ammonium bromide, decyl triethyl ammonium chloride, decyl dimethyl hydroxyethyl ammonium chloride, decyl dimethyl hydroxyethyl ammonium chloride bromide, C_{12-15}dimethyl hydroxyethyl ammonium chloride, C_{12-15}dimethyl hydroxyethyl ammonium chloride bromide, coconut dimethyl hydroxyethyl ammonium chloride, coconut dimethyl hydroxyethyl ammonium bromide, myristyl trimethyl ammonium methyl sulphate, lauryl dimethyl ben-

zyl ammonium chloride, lauryl dimethyl benzyl ammonium bromide, lauryl dimethyl(ethenoxy)$_4$ ammonium chloride, lauryl dimethyl(etbenoxy)$_4$ ammonium bromide, N-alkyl(C_{12-18})dimethylbenzyl ammonium chloride, N-alkyl(C_{14-18})dimethyl-benzyl ammonium chloride, N-tetradecylidmethylbenzyl ammonium chloride monohydrate, dimethyl didecyl ammonium chloride, N-alkyl and (C_{12-14})dimethyl 1-naphthylmethyl ammonium chloride, trimethylammonium halide, alkyl-trimethylammonium salts, dialkyl-dimethylammonium salts, lauryl trimetbyl ammonium chloride, ethoxylated alkyamidoalkyldialkylammonium salt, an ethoxylated trialkyl ammonium salt, dialkylbenzene dialkylammonium chloride, N-didecyldimelhyl ammonium chloride, N-tetradecyldimetbylbenzyl ammonium, chloride monohydrate, N-alkyl (C_{12-14})dimethyl 1-naphthylmethyl ammonium chloride, dodecyldimetbylbenzyl ammonium chloride, dialkyl benzenealkyl ammonium chloride, lauryl trimethyl ammonium cbloride, alkylhenzyl methyl ammonium chloride, alkyl benzyl dimethyl ammonium bromide, C_{12} trimethyl ammonium bromides, C_{15} trimethyl ammonium bromides, C_{17} trimethyl ammonium bromides, dodecylbenzyl triethyl ammonium chloride, poly-diallyldimethylammonium chloride (DADMAC), dimethyl ammouium chlorides, alkyldimetbylammonium balogenides, tricetyl methyl ammonium chloride, decyltrimethylammonium bromide, dodecyltrielhylammonium bromide, tetradecyltrimethylammonium bromide, methyl trioctylammonium chloride, POLYQUAT 10™, tetrahutylammonium bromide, benzyl trimethylammonium bromide, choline esters, henzalkonium chloride, stearalkonium chloride compounds, cetyl pyridinium bromide, cetyl pyridinium chloride, halide salts of quatemized polyoxyethylalkylamines, MIRAPOL™, ALKAQUAT™, alkyl pyridinium salts; amines, amine salts, amine oxides, imide azolinium salts, protonated quaternary acrylamides, methylated quaternary polymers, and cationic guar.

58. The method of any of claims **54**, **56**, or **57**, wherein the composition is bioadhesive.

59. The method of claim 42, comprising as a surfaee stabilizer Tween® 80, Tetronic® T908, dioctylsulfosuccinate, polyvinyl acetate, Pluronic® 127, or a mixture thereof.

60. A method of treating a subject in need comprising administering to the subject an effective amount of a composition comprising:

(a) particles of a griseofulvin or a salt thereof, wherein the griseofulvin particles have an effective average particle size of less than about 2000 nm; and

(b) at least one surface slabilizer, wherein the surface stabilizer is not a polyalkylene block copolymer which gels at physiological temperature, and wbich contains one or more polyoxyethylene blocks and one or more polyoxy (higher alkylene) blocks, wherein at least some of the blocks are linked together by an oxymethylene group.

61. The method of claim 60, wherein the griseofulvin or a salt tbereof is selected from the group consisting of a crystalline phase, an amorphous phase, a semi-crystalline phase, a semi-amorphous phase, and mixtures thereof.

62. The method of claim 60, wherein tbe effective average particle size of the griseofulvin particles is selected from tbe group consisting of less than about 1900 nm, less than about 1800 nm, less than about 1700 nm, less than about 1600 nm, less than about 1500 nm, less than about 1400 nm, less than about 1300 nm, less than about 1200 nm, less than about 1100 nm, less than about 1000 nm, less than about 900 nm, less than about 800 nm, less than about 700 nm, less than about 600 nm, less than about 500 nm, less than about 400 nm, less than about 300 nm, less than about 250 nm, less than about 200 nm, less than about 100 nm, less than about 75 nm, and less tban about 50 nm.

63. The method of claim 60, wherein the composition is formulated for administration selected from the group consisting of oral, pulmonary, rectal, opthalmic, colonic, parenteral, intracisternal, intravaginal, intraperitoneal, local, buccal, nasal, and topical administration

64. The method of claim 60, wherein the composition is a dosage form selected from the group consisting of liquid dispersions, oral suspensions, gels, aerosols, ointments, creams, controlled release formulalions, fast melt formulations, lyophilized formulations, tablets, capsules, delayed release formulations, extended release formulations, pulsatile release formulalions, and mixed immediate release and controlled release formulations.

65. The method of claim 60, wherein the composition further comprises one or more pharmaceutically acceptable excipients, carriers, or a combination thereof.

66. The method of claim 60, wherein griseofulvin or a salt thereof is present in an amount selected from the group consisting of from about 99.5% to about 0.001%, from about 95% to about 0.1%, and from about 90% to about 0.5%, hy weight, hased on the total combined dry weight of the griseofulvin or a salt thereof and at least one surface stabilizer, not including other excipients.

67. The method of claim 60, wherein the at least one surface stabilizer is present in an amount selected from the group consisting of from about 0.5% to about 99.999% by weight, from about 5.0% to about 99.9% hy weight, and from about 10% to about 99.5% hy weight, hased on the total combined dry weight of the griseofulvin or a salt thereof and at least one surface stabilizer, not including other excipients.

68. The method of claim 60, utilizing at least two surface stabilizers.

69. The method of claim 60, wherein the surface stabilizer is selected from the group consisting of an anionic surface stabilizer, a cationic surface stabilizer, a zwitterionic surface stabilizer, and an ionic surface stabilizer.

70. The method of claim 69, wherein the at least one surface stabilizer is selected from the group consisting of cetyl pyridinium chloride, gelatin, casein, phosphatides, dextran, glycerol, gum acacia, cholesterol, tragacanth, stearic acid, benzalkonium chloride, calcium stearate, glycerol monostearate, cetostearyl alcohol, cetomacrogol emulsifying wax, sorbitan esters, polyoxyethylene alkyl ethers, polyoxyethylene castor oil derivatives, polyoxyethylene sorbitan fatty acid esters, polyethylene glycols, dodecyl trimethyl ammonium bromide, polyoxyethylene stearates, colloidal silicon dioxide, phosphates, sodium dodecylsulfate, carboxymethylcellulose calcium, hydroxypropyl celluloses, hypromellose, carboxymethylcellulose sodium, methylcellulose, hydroxyethylcellulose, hypromellose phthalate, noncrystalline cellulose, magnesium aluminum silicate, triethanolamine, polyvinyl alcobol, polyvinylpyrrohidone, 4-(1,1,3,3-tetramethylbutyl)-phenol polymer with ethylene oxide and formaldehyde, poloxamers; poloxamines, a charged phospholipid, dioctylsulfosuccinate, dialkylesters of sodium sulfosuccinic acid, sodium lauryl sulfate, alkyl aryl poly-

ether sulfonates, mixtures of sucrose stearate and sucrose distearate, p-isononylphenoxypoly-(glycidol), decanoyl-N-methylglucamide, n-decyl ,β-D-glucopyranoside; n-decyl β-D-maltopyranoside; n-dodecyl β-D-glucopyranoside; n-dodecyl β-D-maltoside, heptanoyl-N-methylglucamide; n-heptyl-β-D-glucopyranoside; n-heptyl ,β-D-thiogtucoside, n-hexyl β-D-glucopyranoside; nonanoyl-N-methylglucamide; n-noyl β-D-glucopyranoside; octanoyl-N-methylglucamide, n-octyl-,β-D-glucopyranoside; octyl β-D-thioglucopyranoside; lysozyme, PEG-phospholipid, PEG-cholesterol, PEG-cholesterol derivative, PEG-vitamin A, PEG-vitamin E, and random copolymers of vinyl acetate and vinyl pyrrolidone.

71. The method of claim 69, wherein the at least one cationic surface stahilizer is selected from the group consisting of a polymer, a biopolymer, a polysaccbaride, a cellulosic, an alginate, a nonpolymeric compound, and a phospholipid.

72. The method of claim 69, wherein the surface stabilizer is selected from the gmup consisting of benzalkonium chloride, polymethylmethacrylate trimetbylammonium bromide, polyvinylpyrrolidone-2-dimethylaminoetbyl methacrylate dimethyl sulfate, hexadecyltrimethyl ammonium hmmide, cationic lipids, sulfonium compounds, phosphonium compounds, quarternary ammonium compounds, benzyl-di(2-chlomethyl) ethylammonium bromide, coconut trimethyl ammonium chloride, coconut trimethyl ammonium hromide, coconut methyl dihydroxyethyl ammonium chloride, coconut methyl dihydroxyethyl ammonium bromide, decyl triethyl ammonium chloride, decyl dimethyl hydroxyethyl ammonium chloride, decyl dimethyl hydroxyethyl ammonium chloride hromide, C_{12-15}dimethyl hydroxyethyl ammonium chloride, C_{12-15}dimethyl hydroxyethyl ammonium chloride bromide, coconut dimethyl hydroxyethyl ammonium chloride, coconut dimethyl bydroxyethyl ammonium bromide, myristyl trimethyl ammonium metbyl sulphate, lauryl dimethyl benzyl ammonium chloride, lauryl dimethyl benzyl ammonium hmmide, lauryl dimethyl-(ethenoxy)$_4$ ammonium chloride, lauryl dimethyl(ethenoxy)$_4$ ammonium bromide, N-alkyl(C_{12-18})dimethylbenzyl ammonium chloride, N-alkyl(C_{14-18})dimethyl-benzyl ammonium chloride, N-tetradecylidmethylbenxyl ammonium chloride monohydrate, dimethyl didecyl ammonium chloride, N-alkyl and (C_{12-14})dimethyl 1-naphthylmethyl ammonium chloride, trimethylammonium halide, alkyl-trimethylammonium salts, dialkyl-dimethylammonium salts, lauryl trimethyl amnionium chloride, ethoxylated alkyamidoalkyldialkylammonium salt, an ethoxylated trialkyl ammonium salt, dialkylbenzene dialkylammonium chloride, N-didecyldimethyl ammonium chloride, N-tetradeeyldimethylbenzyl ammonium, chloride monohydrate, N-alkyl(C_{12-14})dimethyl 1-naphthylmethyl ammonium chloride, dodecyldimethylbenzyl ammonium chloride, dialkyl benzenealkyl ammonium chloride, lauryl trimethyl ammonium chloride, alkylbenzyl methyl ammonium chloride, alkyl benzyl dimetbyl ammonium bromide, C_{12} trimethyl ammonium bromides, C_{15} trimethyl ammonium bromides, C_{17} trimethyl ammonium bromides, dodecylbenzyl triethyl ammonium chloride, poly-diallyldimethylammonium chloride (DADMAC), dimethyl ammonium chlorides, alkyldimethylammonium halogenides, tricetyl methyl ammonium chloride, decyltrimethylammonium bromide, dodecyltriethylammonium bromide, tetradecyltrimethylammonium hromide, methyl trioctylammonium chloride, POLYQUAT 10™, tetrabutylammonium bromide, benzyl trimethylammonium bromide, choline esters, benzalkonium chloride, stearalkonium chloride compounds, cetyl pyridinium bromide, cetyl pyridinium chloride, halide salts of quatemized polyoxyethylalkylamines, MIRAPOL™, ALKAQUAT™, alkyl pyridinium salts; amines, amine salts, amine oxides, imide azolinium salts, protonated quatemary acrylamides, methylated quatemary polymers, and cationic guar

73. The method of any of claims 69, 71, or 72, wherein the composition is bioadhesive.

74. The method of claim 60, comprising utilizing as a surface stabilizer Tween® 80, Tetronic® T908, dioctylsulfosuccinate, polyvinyl acetate, Pluronic® F127, or a mixture thereof.

75. The method of claim 60, additionally comprising administering one or more non-griseofulvin active agents.

76. The method of claim 75, wherein said additional one or more non-griseofulvin active agents are selected from the group consisting of nutraceuticals, amino acids, proteins, peptides, nucleotides, anti-obesity drugs, central nervous system stimulants, carotenoids, corticosteroids, elastase inhibitors, anti-fungals, oncology therapies, anti-emetics, analgesics, cardiovascular agents, anti-inflammatory agents, anthelmintics, anti-arrhythmic agents, antibiotics, anticoagulants, antidepressants, antidiabetic agents, antiepileptics, antihistamines, antihypertensive agents, antimuscarinic agents, antimycobacterial agents, antineoplastic agents, immunosuppressants, antithymid agents, antiviral agents, anxiolytics, sedatives, astringents, alpha-adrenergic receptor blocking agents, beta-adrenoceptor blocking agents, blood products, blood substitutes, cardiac inotropic agents, contrast media, corticosteroids, cough suppressants, diagnostic agents, diagnostic imaging agents, diuretics, dopaminergics, haemostatics, immunological agents, lipid regulating agents, muscle relaxants, parasympathomimetics, parathyroid calcitonin, parathyroid biphosphonates, prostaglandins, radiopharmaceuticals, sex hormones, anti-allergic agents, stimulants, anoretics, sympathomimetics, thyroid agents, vasodilators, and xanthines.

77. The method of claim 75, wherein said at least one additional non-griseofulvin active agent is an anti-fungal agent.

78. The method of claim 75, wherein at least one additional non-griseofulvin active agent is vitamin E.

79. The method of claim 60, wherein the T_{max} of the griseofulvin, when assayed in the plasma of a mammalian subject following administration, is less than the T_{max} for a non-nanoparticulate griseofulvin formulation, administered at the same dosage.

80. The method of claim 79, wherein the T_{max} is selected from the group consisting of not greater than about 90%, not greater than about 80%, not greater than about 70%, not greater than about 60%, not greater than about 50%, not greater than about 30%, not greater than about 25%, not greater than about 20%, not greater than about 15%, not greater than about 10%, and not greater than about 5% of the T_{max} exhibited by a non-nanoparticulate griseofulvin formulation, administered at the same dosage.

81. The method of claim 60, wherein the C_{max} of the griseofulvin, when assayed in the plasma of a mammalian subject following administration, is greater than the C_{max} for a non-nanoparticulate griseofulvin formulation, administered at the same dosage.

82. The method of claim 81, wherein the C_{max} is selected from the group consisting of at least about 50%, at least about 100%, at least about 200%, at least about 300%, at least about 400%, at least about 500%, at least about 600%, at least about 700%, at least about 800%, at least about 900%, at least about 1000%, at least about 1100%, at least about 1200%, at least about 1300%, at least about 1400%, at least about 1500%, at least about 1600%, at least about 1700%, at least about 1800%, or at least about 1900% greater than the C_{max} exhibited by a non-nanoparticulate formulation of griseofulvin, administered at the same dosage.

83. The method of claim 60, wherein the AUC of the griseofulvin, when assayed in the plasma of a mammalian subject following administration, is greater than the AUC for a non-nanoparticulate griseofulvin formulation, administered at the same dosage.

84. The method of claim 83, wherein the AUC is selected from the group consisting of at least about 25%, at least about 50%, at least about 75%, at least about 100%, at least about 125%, at least about 150%, at least about 175%, at least about 200%, at least about 225%, at least about 250%, at least about 275%, at least about 300%, at least about 350%, at least about 400%, at least about 450%, at least about 500%, at least about 550%, at least about 600%, at least about 750%, at least about 700%, at least about 750%, at least about 800%, at least about 850%, at least about 900%, at least about 950%, at least about 1000%, at least about 1050%, at least about 1100%, at least about 1150%, or at least about 1200% greater than the AUC exhibited by the non-nanoparticulate formulation of griseofulvin, administered at the same dosage.

85. The method of claim 60, wherein the griseofulvin composition does not produce significantly different absorption levels when administered under fed as comPared to fasting conditions.

86. The method of claim 85, wherein the difference in absorption of the griseofulvin composition of the invention, when administered in the fed versus the fasted state, is selected from the group consisting of less than about 100%, less than about 90%, less than about 80%, less than about 70%, less than about 60%, less than about 50%, less than about 40%, less than about 30%, less than about 25%, less than about 20%, less than about 15%, less than about 10%, less than about 5%, and less than about 3%.

87. The method of claim 60, wherein administration of the composition to a human in a fasted state is bioequivalent to administration of the composition to a human in a fed state.

88. The method of claim 87, wherein "bioequivalency" is established by a 90% Confidence Interval of between 0.80 and 1.25 for both C_{max} and AUC.

89. The method of claim 87, wherein "bioequivalency" is established by a 90% Confidence Interval of between 0.80 and 1.25 for AUC and a 90% Confidence Interval of between 0.70 to 1.43 for C_{max}.

90. The method of claim 60, wherein the subject is a human.

91. The method of claim 60, used to treat a condition selected from the group consisting of dermatophyte infections and ringworm infections.

92. The method of claim 91, used to treat a condition selected from the group consisting of ringworm infections of the scalp, hair, nails, and skin.

93. The method of claim 60, used to treat a condition selected from the group consisting of *Tinea capitis* (ringworm of the scalp), *Tinea corporis* (ringworm of the body), *Tinea pedis* (athlete's foot), *Tinea unguium* (ringworm of the nails), *Tinea cruris* (ringworm of the thigh), and *Tinea barbae* (barber's itch).

94. The method of claim 60, used to treat a fingal infection of an organism selected from the group consisting of *Trichophyton rubrum, Trichophyton tonsurans, Trichophyton mentagrophytes, Trichophyton interdigitalis, Trichophyton verrucosum, Trichophyton sulphureum, Trichophyton schoenleini, Trichophyton audouini, Trichophyton canis, Trichophyton gypseum, Trichophytonfloccosum, Trichophyton megnini, Trichophyton gallinae,* and *Trichophyton crateriform.*

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